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# BRITISH MEDICAL BULLETIN

Vol. 2 (1944) No. 1

## PENICILLIN 1929–1943

### SPECIAL CONTRIBUTIONS

PENICILLIN : ITS PROPERTIES AND POWERS AS A THERAPEUTIC AGENT
by Professor L. P. Garrod ... 2

THE DISCOVERY OF PENICILLIN
by Professor A. Fleming ... 4

THE DISCOVERY OF THE CHEMOTHERAPEUTIC PROPERTIES OF PENICILLIN
by Dr. E. Chain & Professor H. W. Florey ... 5

PENICILLIN FOR SELECTIVE CULTURE AND FOR DEMONSTRATING BACTERIAL INHIBITIONS
by Professor A. Fleming ... 7

OTHER ANTIBACTERIAL SUBSTANCES FROM BACTERIA AND MOULDS
by Dr. E. Chain ... 8

CLINICAL USES OF PENICILLIN
by Dr. M. E. Florey ... 9

### REVIEW OF SELECTED PAPERS

**Discovery : Early Investigations : Bacteriological Applications**

1929 First Description ; Production : Selective Bacteriostasis ... 13
1930 Use for Isolation of *H. Influenzae* (Pfeiffer) ... 14
1932 Uses in Bacteriology (and as a Wound Dressing) ... 14
1932 Observations on Production and Extraction ... 15
1937 Use for Isolation of *H. pertussis* ... 15
1942 Use for Isolation of Acne Bacillus ... 15

**Chemotherapeutic Action : Pharmacology : Bacteriological Investigations**

1940 First Cure of Experimentally Infected Animals : Preliminary Report on Pharmacology ... 15
1940 Inactivation by a Bacterial Enzyme—Penicillinase ... 16
1940 Morphological Effects on Bacteria ... 16

**First Clinical Trials : Production : Biological Properties**

1941 Successful Trials in Human Infections : Laboratory Large-Scale Production : Assay : Extraction : Antibacterial Action : Toxicity : Pharmacology ... 16

**Further Chemotherapeutic, Bacteriological, and other Investigations**

1942 Biological Properties of Purer Samples : Calcium Salt ... 18
1942 Methods of Testing Bacteriostasis ... 18
1942-3 Further Experimental Clostridial Infections ... 19
1943 Experimental Staphylococcal Lesions of the Eye ... 19
1943 Experimental Pneumococcal Lesions of the Eye ... 19
1943 Negative Experiments with Viruses ... 20
1943 Synergistic Effect of Para-aminobenzoic Acid and Sulphapyridine ... 20
1943 Inhibitory Action on Urease ... 20
1943 Production : Methods of Increasing the Yield ... 20
1943 Rapid Method for Assay ... 20
1943 Elimination from Culture Media by Penicillinase ... 21
1943 Effect on Tissues ... 21

**Purification and Investigation of Chemical Properties**

1942 Purification and Some Physical and Chemical Properties ... 21
1942 Spectrographic Examination of Preparations ... 21
1942 Purification and Chemistry ... 22
1943 Penicillamine, a Degradation Product ... 22
1943 Penillic Acid ... 22

**Further Clinical Trials**

1941 Local Administration in Nasal Carriers of Staphylococci ... 22
1943 Methods and Rationale of Local and Systemic Administration in a Series of Cases ... 23
1943 Elimination of Streptococci and Staphylococci from Burns ... 24
1943 Local Treatment of War Wounds ... 24
1943 Detailed Report on Intrathecal Administration in a Case of Streptococcal Meningitis ... 24
1943 Treatment of War Wounds in North Africa ... 25
1943 Infected Burns and Surface Wounds ... 26

### APPENDIX

The Oxford Unit ... 26
The Slide-Cell Technique ... 26
Communications Published in Britain but not Reviewed in this Number ... 26
Communications Published in Other Countries ... 27

*Interest in penicillin is becoming world-wide, but war-time disturbances of communications have added to the difficulty in some countries of obtaining access to the relevant literature, which covers a span of 15 years and is distributed among journals of divergent character.*

*These circumstances seemed to indicate that a useful purpose might be served by a special number of this Bulletin devoted entirely to a survey of penicillin, from the date of its discovery to the close of 1943—the year in which clinical trials on a more adequate scale became possible.*

*Acknowledgment is due to the authors who have found the time—in some cases at very short notice—to contribute articles to this number.*

*Professor L. P. Garrod has already been the subject of a note (BMB 48) in this Bulletin. He is a member of the Penicillin Therapeutic Trials Committee of the Medical Research Council, and has for many years been studying the chemical control of bacterial infections. His writings on this subject reveal a rare gift of lucid exposition.*

*Professor Alexander Fleming, the discoverer of penicillin, has been occupied in the study of chemical antibacterial agents and their effect on the normal defences of the body since the war of 1914–18. He has also been deeply interested in certain physiological antibacterial agents, such as lysozyme, and in bacterial antagonisms. He is Professor of Bacteriology in the University of London and Assistant Director of the Inoculation Department of St. Mary's Hospital. In 1943 he was elected a Fellow of the Royal Society.*

*Professor H. W. Florey graduated at Adelaide University, Australia, in 1921 and came to England as a Rhodes Scholar in 1922. He has done original work in various fields, notably on the mechanism of inflammatory changes, and has travelled widely. In 1935 he was elected Professor of Pathology at Oxford. In more recent years his attention has been increasingly directed to the study of bacterial inhibition, and it was this interest in the general problem which led him, with his Oxford team of scientists, to discover that penicillin can be purified and used as a chemotherapeutic agent. Professor Florey became a Fellow of the Royal Society in 1941.*

*Dr. E. Chain is University Demonstrator in Chemical Pathology in the Sir William Dunn School of Pathology, Oxford. He has published many papers on various biochemical problems. His chief interests have been the study of enzymes and the isolation of physiologically active substances from natural sources. He has been closely concerned throughout with chemical and biochemical work on penicillin.*

*Dr. M. E. Florey is, like her husband, a medical graduate of Adelaide University. She played a leading part in the early clinical trials at Oxford, and has since made a detailed study of the effects of penicillin applied locally. She is at present engaged in exploring fresh fields of clinical application.*

*Acknowledgment is due to Dr. E. P. Abraham, Dr. M. A. Jennings and Dr. A. G. Sanders, all of the School of Pathology at Oxford, for the preparation of abstracts for the "Review of Selected Papers." Dr. Jennings, in addition to contributing many abstracts and providing references for the bibliographies, has given much valuable help at all stages of the preparation of this number.*

# SPECIAL CONTRIBUTIONS



## PENICILLIN: ITS PROPERTIES AND POWERS AS A THERAPEUTIC AGENT

LAWRENCE P. GARROD, M.D., F.R.C.P.

*from the Department of Pathology, St. Bartholomew's Hospital, London*

The chemotherapy of bacterial infections, which had been little more than an ideal until 1935, became a reality with the advent of prontosil. During the rapid development of sulphonamide treatment which followed, other organisms than *Streptococcus pyogenes* were found to be susceptible, and successful attacks on *Gonococcus*, *Meningococcus* and *Pneumococcus* followed in swift succession. It seemed likely at one time that with the advent of new drugs of this type all bacterial infections could be brought under control. This hope has been disappointed, and for some years we have been discovering the limitations of sulphonamide treatment rather than extending its scope. Even among the most susceptible bacteria some strains occur with exceptional resistance to the sulphonamide drugs, while there are many species relatively or wholly resistant to them.

Another great therapeutic discovery has now been made, which provides a remedy for some of the infections in which sulphonamides fail. That it will do in certain cases what these drugs will not is only part of its claim to our interest: it is a substance with hitherto unheard-of—almost unimagined—properties. It combines enormous antiseptic power with such a degree of freedom from toxicity to the mammalian body that one thousand times the concentration necessary for therapeutic action can be produced in the blood without ill effect. Such a combination of deadliness to bacteria with harmlessness to the body is more than the most sanguine chemotherapist can have pictured as possible before the properties of penicillin became known. Treatment with it is governed not by the fear of over-dosage, but only by anxiety to employ so precious a remedy with the utmost possible economy.

The two principal stages in the evolution of penicillin as a therapeutic agent are described in succeeding articles by the discoverers themselves, Professor Alexander Fleming, of St. Mary's Hospital, London, and Dr. E. Chain and Professor H. W. Florey, of the University of Oxford. In other articles in this number Professor Fleming describes the use of penicillin in bacteriological techniques, Dr. Chain refers to other antibiotics, and Dr. M. E. Florey reviews the clinical uses of penicillin.

It is my task to present a general picture of what penicillin now is and what it will do, as an introduction to the more detailed clinical contributions which follow. Penicillin is now being produced on a considerable and rapidly increasing scale, in both England and the U.S.A., by extraction from mass cultures of *Penicillium notatum*. No other method of production is yet known, although synthesis is an eventual possibility. In each of these countries the output has been officially controlled, and supplies have been afforded for clinical trials only to chosen investigators. In England not only the original employment of penicillin as a therapeutic agent but much of the subsequent study on which our present knowledge is based has been due to the enterprise of Florey himself and his colleagues.

### The Properties of Penicillin

Penicillin is an unstable acid, and the preparations used in therapeutics are its salts. The sodium salt employed for systemic treatment is hygroscopic and somewhat less stable than the more easily handled calcium salt, which is used mainly for local application. Potency is expressed in Florey[1] [Oxford] units, an arbitrary amount determined by comparison with a standard preparation. Pure penicillin would have a potency of at least 1,000 units per mg.; that in present use is far from pure—owing to the serious loss of active substance which further purification entails—and material with a potency of 100 units or less per mg. is quite satisfactory for clinical use. It has been shown experimentally that an increase in purity diminishes toxicity, and clinical experience has shown that untoward effects such as pain on intramuscular injection and fever or thrombophlebitis following intravenous administration are caused mainly by products of low potency. These effects are thus due to impurities rather than to penicillin itself.

In the presence of penicillin, even in very low concentration, certain species of bacteria not only cannot multiply but slowly die. Whether this effect is "bactericidal" or purely "bacteriostatic" is not clear; the distinction is not easily

[1] [*see BMB* 239]

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made, and the mechanism of the effect is unknown, although the peculiar changes in bacterial morphology first observed by Gardner [1] suggest that at least the process of division is inhibited. More important from the practical standpoint is the fact that this effect is exerted as well in serum, blood, or even pus, as in a simple medium such as broth. Within wide limits it is also independent of the number of bacteria present. Yet even very high concentrations are without effect on the activity of leucocytes: both by this form of study and by several others penicillin has been shown to have almost no local tissue toxicity. These facts explain the superiority of penicillin over sulphonamides for direct application to wounds; concentrated sulphonamides are by no means altogether non-toxic, they are far from indifferent to bacterial numbers, acting best when only few are present, and they are inhibited by the breakdown products in pus. Penicillin overcomes all these difficulties, and the consequent difference in effect is fully equal to expectation.

It is essential to understand that penicillin exerts this action only on certain species of bacteria; it is indeed the most highly selective antiseptic known, and for years was used by Fleming as an agent in selective culture media, which prevented the growth of some bacteria and permitted that of others. Most of the susceptible species are gram-positive; they include the three main pyogenic cocci (*Staphylococcus*, *Pneumococcus* and *Streptococcus pyogenes*), the gas gangrene group (among which *Cl. œdematiens*, although resistant to sulphonamides, is almost as susceptible as *Cl. welchii* to penicillin), *B. anthracis* and *C. diphtheriæ*. The only fully susceptible gram-negative species are *Neisseriæ*, the gonococcus and meningococcus. Among resistant organisms are the tubercle bacillus and almost all gram-negative bacilli, including the typhoid-dysentery group (some of which are slightly sensitive), the genera *Brucella* and *Hæmophilus*, and two frequent wound invaders, *Proteus* and *Ps. pyocyanea*.

### Systemic Penicillin Treatment

Penicillin can be used therapeutically in two ways: It can be applied locally, or administered by parenteral injection so that it circulates in the blood and reaches every part of the body. The former method is economical but often difficult and sometimes inapplicable, the latter sure in its effect but immensely costly, using as a rule at least fifty times the amount needed for local treatment. Penicillin is absorbed from the alimentary tract, but cannot be given by this route because much of it is destroyed by acid in the stomach or by bacteria during rectal infusion. It must therefore be injected, either intramuscularly or intravenously. Unfortunately it is rapidly excreted in the urine, and the maintenance of an adequate blood level has been aptly compared by Florey to an effort to keep a bath full with the plug out. Such a level can, however, be maintained by continuous intravenous infusion or by intramuscular injections at intervals of not more than three hours day and night, the daily dose for an adult being about 120,000 units. This may have to be continued for seven days or even longer. A sudden and dramatic improvement is rarely seen, and sustained treatment, arduous for those in charge and disagreeable for the patient, is the price of success.

In present circumstances it is unjustifiable to administer penicillin systemically for any condition amenable to sulphonamide treatment. Septicæmia due to *Strept. pyogenes* or *Pneumococcus* is therefore treated only in the exceptional cases found to be sulphonamide-resistant. Staphylococcal septicæmia is always relatively resistant to sulphonamides, and penicillin, when available, is more decidedly indicated for this condition than for any other. Apart from septicæmia, extensive and deep-seated infections inaccessible by local applications require systemic treatment; these include osteomyelitis, severe cellulitis and gas gangrene. It has recently been shown by Florey and Cairns in battle casualties from Sicily that potentially infected compound fractures can be closed with the aid of this treatment. American experience suggests that sulphonamide-resistant pneumococcal pneumonia will respond to a very short course, and observations made in both the American and British armies have shown that cases of gonorrhœa can be cured by a total dose of little more than 100,000 units given in a space of about 24 hours. With more experience and larger supplies, further and more precise indications for this form of administration will doubtless be obtained.

[1] [*see BMB* 211]

### Local Penicillin Treatment

The local application of penicillin takes many forms, some calling for ingenuity which is well rewarded by the remarkable effects to be obtained at little cost. Application to burns and other superficial and accessible wounds is secured by a cream or powder, the only satisfactory diluent known for the latter being sulphanilamide. These preparations can be relied on to eliminate infection by hæmolytic streptococci and staphylococci from such areas. Similar applications are highly successful in the treatment of skin infections such as impetigo and sycosis barbæ. The treatment of deeper wounds demands arrangements whereby a preparation can be enabled to penetrate them completely and persist there. A radical change in surgical technique is often necessary to secure this. Thus an abscess cavity or other infected area which would normally be laid widely open and drained freely may either not be incised at all but treated by aspirations and injection of penicillin solution, or if incised, it may be sutured again and closed, except for a small aperture containing a tube through which the solution is introduced at intervals afterwards.

The various applications of this principle are too numerous to detail here. Historically the first was the modified operation which enabled the Floreys to treat mastoiditis, and the latest, and at present the most important, is the closure of recent soft-tissue battle wounds advocated by Florey and Cairns on the strength of their recent experience in North Africa. Much more remains to be done in devising methods of using penicillin to good effect locally in the infinite variety of wounds, sinuses and other lesions to which it can be applied.

Common causes of failure are morbid anatomical conditions such that the solution either does not reach all parts of the lesion or does not persist there, the presence of bacteria which are resistant to penicillin or actually destroy it, and antecedent fibrosis mechanically preventing closure and healing.

A special example of local treatment is the intrathecal injection of penicillin solution for the treatment of meningitis. This has been highly successful in a few cases, and is imperative for treating this condition, since penicillin, unlike the sulphonamides, does not pass freely from the blood into the cerebrospinal fluid.

### Future Prospects

The time may not be very far distant when penicillin will become generally available, at least in limited quantities. This will certainly happen soon after the war is over, when military surgery, which now has priority over other demands, no longer calls for a large supply. It is therefore perhaps not too early to issue a warning that the successful use of penicillin is by no means as easy as treatment with sulphonamides. It has so far been exclusively in the hands of experts under research conditions, who have obtained good results only by unremitting care and with the aid of strict laboratory control. The duties of the laboratory in connection with penicillin treatment are very onerous. It is necessary first to determine the nature of the infection and the sensitivity to penicillin of the organisms concerned. Local treatment should then be controlled by frequent further cultures. Systemic treatment calls for repeated estimations of the penicillin content of the blood in order to verify adequate dosage. Owing to its instability and liability to contamination with resistant bacteria, penicillin should also be dispensed by the bacteriologist rather than the pharmacist. Without such services in addition to expert surgical and nursing care, any but the simplest forms of penicillin treatment may easily fail.

Looking further into the future, it may be asked what prospects there are of extending the scope of this treatment. The full possibilities of penicillin itself have not yet been explored, even in infection by bacteria known to be susceptible. For example, little is yet known about its effect in gas gangrene, and still less about syphilis, diphtheria or anthrax. But is there any possibility that substances related to penicillin will be found which attack bacteria on which penicillin has little or no action? A systematic study of other moulds has been in progress for several years, and so far none has been found which produces an antibacterial substance equal in therapeutic value to penicillin, although hundreds of species have been tested. Some of these sub-

stances do, however, act on a wider range of bacteria including gram-negative bacilli. This property has not yet been found uncombined with toxicity to mammalian tissues. The other possible approach is the synthetic. When the structure of penicillin becomes known it may be possible so to vary it that a wider range of activity is secured. One thing quite certain is that penicillin differs fundamentally from other antibacterial agents. Its discovery is an achievement of the first magnitude, of which the ultimate consequences cannot yet be foreseen.



# THE DISCOVERY OF PENICILLIN

ALEXANDER FLEMING, M.B., F.R.C.S., F.R.S.

*from the Inoculation Department, St. Mary's Hospital, London*

I have been asked to say how I came to discover penicillin. After a lapse of fifteen years it is very difficult to say just what processes of thought were involved, but it seems necessary to go back much further than 1928 when the activity of penicillin was first observed.

### Antecedent Investigations

As one of the pupils of Sir Almroth Wright I had naturally been deeply interested during the whole of my career in the destruction of bacteria by leucocytes. During the 1914–18 war I spent much time investigating problems in connexion with septic wounds, and I was then impressed with the antibacterial power of the leucocytes contained in pus which exuded from septic wounds. It was also clear from these investigations that the chemical antiseptics in common use were more destructive on the leucocytes than they were on bacteria.

This interest in antiseptics and leucocytes was continued in post-war years, and in 1924 I was able, by a simple method, to demonstrate clearly the antileucocytic power of antiseptics, and to indicate that if the antileucocytic action of an antiseptic were greater than its antibacterial action, such antiseptic was unlikely to be successful in the treatment of a septic wound.

In 1922 I described lysozyme, a powerful antibacterial ferment occurring naturally in human tissues and secretions, in the white of the domestic hen's egg, and elsewhere.

### Effect of Contamination of a Culture

In September 1928 I was working on the variation of staphylococcus colonies following on a publication by Professor Bigger, who had shown that colonies of widely different appearance could be produced from a pure culture of an ordinary pyogenic staphylococcus. In the course of these observations culture plates of staphylococci were examined at intervals with a dissecting microscope, which involved a temporary removal of the cover and exposure to contamination from the air. After examination, some of the culture plates were placed in the incubator and others were left to mature at room temperature. Further examination of one of the latter showed that a mould colony had developed towards one side of the culture plate. Such contamination with a mould was, in the circumstances, not unexpected, but what was astonishing was that in this particular culture plate the staphylococcal colonies for some considerable distance round the mould growth were obviously undergoing lysis. What had originally been a well-grown staphylococcal colony was now a faint shadow of its former self.

It is certain that every bacteriologist has not once but many times had culture plates contaminated with moulds. It is also probable that some bacteriologists have noticed similar changes to those noted above, but that, in the absence of any special interest in naturally occurring antibacterial substances, the cultures have simply been discarded.

It was, however, fortunate that, with the background I have briefly sketched, I was always on the lookout for new bacterial inhibitors, and when I noticed on a culture plate that the staphylococcal colonies in the neighbourhood of a mould had faded away I was sufficiently interested in the antibacterial substance produced by the mould to pursue the subject.

### Experimental Observations

The next step was to touch the mould colony with a platinum wire and transfer some spores to a culture tube of Sabouraud's medium which, to the ordinary bacteriologist, is the usual medium for growing moulds. It is interesting that until recently all the penicillin used clinically had been produced from sub-cultures of this original tube. This first pure culture of the mould has not survived the years, but the original culture plate with the mould colony inducing dissolution of staphylococcal colonies still exists [see Fig. I]. The preservation of this culture shows that, although I was unable to concentrate the antiseptic substance sufficiently for therapeutic use, I yet considered the culture a memorable one.

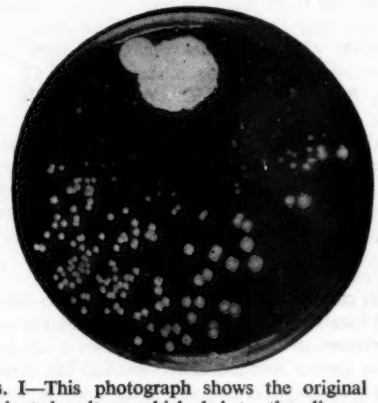


FIG. I—This photograph shows the original contaminated culture which led to the discovery of penicillin. The patch at the top is the growth of mould (*Penicillium notatum*). In the lower third, normal staphylococcal colonies are seen. For some distance around the mould, the growth of staphylococci has been suppressed.

Until recently, all penicillin produced in Britain and the U.S.A. was derived from sub-cultures of the mould colony shown in this photograph (which is reproduced from Fleming's 1929 paper in the *British Journal of Experimental Pathology*).

Having got a pure culture, I grew it in the ordinary nutrient broth used by bacteriologists. It grew as a felted mass on the surface. After a week it was found that the culture fluid diluted some 500 to 800 times would completely inhibit the growth of staphylococci, and it was therefore some two or three times as strong in that respect as pure carbolic acid. It was obvious from this that the antibacterial substance produced by the mould was a remarkable one and demanded further investigation.

The mould belonged to the genus *Penicillium*, so that the active substance, which was then (and still is at the time of writing) of unknown chemical constitution, was christened "penicillin." The mould was later identified as *Penicillium notatum*, a species which had been found by Westling in decaying hyssop in Norway (Thom).

From the appearance of the original plate it was obvious that penicillin was readily diffusible in agar, just as was lysozyme, which I had investigated some six years earlier. The technique which we had used with lysozyme was applicable to penicillin. One method used and figured in my original paper was to cut out a strip of agar from a culture plate, plant various bacteria in streaks at right angles to the gutter thus formed, and then fill the gutter with agar mixed with penicillin. The active substance diffused into the agar and inhibited the different bacteria for a distance varying with their sensitivity to penicillin. Some, such as *B. coli* or

*H. influenzae*, were not inhibited at all, while others, such as *Staphylococcus*, *Streptococcus*, *Pneumococcus*, *Gonococcus*, and the diphtheria bacillus would not grow anywhere near the penicillin strip [see Fig. II]. It was therefore clear that

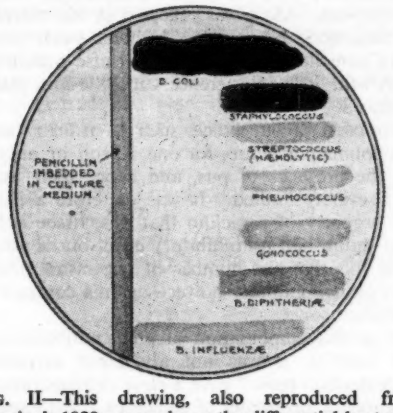



FIG. II—This drawing, also reproduced from Fleming's 1929 paper, shows the differential bacteriostatic effect of a penicillin-containing fluid in a gutter made by cutting out a strip from the agar medium. Inocula of various bacteria have been made at right angles to the gutter. Growth of *B. coli* and *H. influenzae* is not inhibited at all, while growth of the other organisms is inhibited in varying degrees.

penicillin had a specific action on some bacteria and did not affect others, and it is interesting that the bacteria originally found to be sensitive in this way to the crude culture fluid are the same as those which have subsequently been found to be affected by concentrated penicillin used clinically.

In view of the results I had previously obtained with the chemical antiseptics in common use, I proceeded to test whether, like these, penicillin was poisonous to human leucocytes. It had no poisonous effect, nor was it toxic when injected into animals.

### Conclusions

Here, then, we had an antiseptic substance which at that time was unique in having a strong inhibitory effect on many of the common bacteria which infect the human body, but which was not toxic to animals or to human leucocytes. Unfortunately it was a very unstable substance, and early attempts to concentrate it had little success. Although some tentative observations on the local antiseptic action of the crude fluid were made with moderate success, its instability and the smaller number of septic cases in hospital in peace time led to its clinical use not being seriously pursued.

The laboratory results, however, together with the few clinical observations, made me state in the summary of my original paper [1] in 1929 that:

> "It may be an efficient antiseptic for application to, or injection into, areas infected with penicillin-sensitive microbes";

and in 1931 in an article [2] on the use of antiseptics:

> "It is quite likely that it, or a chemical of a similar nature, will be used in the treatment of septic wounds."

The words "chemical of a similar nature" were prompted by the thought that some day a chemist would discover the nature of the active principle, synthetize it, and use either that or some modification as a chemotherapeutic agent. That was years before the introduction of the sulphonamides, and at a time when the only effective antibacterial chemotherapy was the treatment of syphilis by modifications of Ehrlich's salvarsan.

I have used penicillin constantly since 1929 for differential culture,[3] but its use for practical therapeutic purposes remained in abeyance until the Oxford workers started their investigations.[4]

[1] [*see BMB* **203**] [2] [*see BMB* **241**] [3] [*described in BMB* **200**] [4] [*described in BMB* **199**]

199

# THE DISCOVERY OF THE CHEMOTHERAPEUTIC PROPERTIES OF PENICILLIN

E. CHAIN, Ph.D., & H. W. FLOREY, M.B., Ph.D., F.R.S.
*from the Sir William Dunn School of Pathology, Oxford*

Professor Fleming has described how he discovered that the mould *Penicillium notatum* produces a substance which inhibits the growth of certain pathogenic bacteria, and the experiments which he performed with this substance. In this article we propose to trace the steps which led to the discovery of the chemotherapeutic properties of penicillin and eventually to its employment in treating diseases in man.

### Planned Investigation of Antibiotics

It may be of interest to know why the work was taken up in Oxford. In 1929 one of us (H. W. F.) started work on an antibacterial substance, lysozyme, which had been discovered by Fleming in 1922. During the 1930's this work was carried on till the enzyme was purified by Roberts (1937) and its substrate was characterised (Epstein & Chain, 1940). During the later part of this work the present writers prepared a plan for the systematic investigation of antibacterial substances produced by micro-organisms. It was thought that these might be chemically and biologically interesting, especially as many of them were active against pathogenic bacteria.

As long ago as 1877, Pasteur & Joubert noticed that the growth of certain air-borne organisms inhibited the growth of the anthrax bacillus, and suggested that this fact might be of importance in therapeutics. Since that time, many instances have been found of the production of a substance by one microbe which inhibits the growth of others. These inhibitions are due to metabolic products, recently termed "antibiotics."

Attempts had been made to utilise these substances in medicine, the most noteworthy being that of Emmerich & Loew (1899) who extracted "pyocyanase" from *Ps. pyocyanea*, and Dubos (1939) who extracted gramicidin, a mixture of two polypeptides, from *B. brevis*.

The Oxford work on antibiotics began to develop in 1938. Of a number of known antibiotics considered, "pyocyanase" and penicillin were chosen for the first investigations. It appeared from reports in the literature that the latter substance was unstable and therefore difficult to extract, but the fact that it was active against a range of important pathogenic organisms weighed the balance in its favour. Moreover, both Fleming (1932) and Clutterbuck, Lovell & Raistrick (1932), had stated that penicillin activity might, under certain conditions, be retained in the culture medium for some weeks. It seemed worth while to see whether appropriate conditions could be found for extracting the substance, so that further examination of both its biochemical and biological properties could be made.

### Physical and Chemical Properties of Penicillin

It was first established that penicillin was an acid which, as the free acid in aqueous solution, was very unstable, but in the form of alkali and alkaline earth salts was stable between *p*H 5 and *p*H 7. Clutterbuck *et al.* had found that penicillin was extracted into ether on shaking with the culture fluid after it had been acidified. When the ether was evaporated, however, the activity was largely lost. This experiment was confirmed, but it was found possible to prepare stable salts of penicillin by shaking the ether solution with dilute aqueous alkali so as to bring the final *p*H to 7. In addition to ether, a number of organic solvents, for example chloroform and amyl acetate, could be used to extract the free acid form of

penicillin. The salts of penicillin were much more soluble in water than in the organic solvents, and therefore penicillin was removed from the organic solvent by about $\frac{1}{5}$ to $\frac{1}{10}$ the volume of alkali solution. A concentration of penicillin was thereby achieved, and by repeating the extraction several times with different solvents and at suitable *p*H, a considerable purification of penicillin and simultaneous reduction of the bulk of liquid was obtained.

The losses of penicillin during these operations were small if the solutions were kept cold throughout. On drying the final aqueous solution *in vacuo* from the frozen state, a preparation of a salt of penicillin was obtained, in powder form, which kept its antibacterial activity unchanged for a long time.

### Methods of Purification

Chemically, however, the preparation was far from pure, containing, as is now known, not more than a small percentage of pure penicillin. The isolation of penicillin in the pure state from this mixture proved a difficult problem because of its instability towards many reagents and the unfavourable solubilities of the free acid and its salts. It was found that penicillin was destroyed by dilute acid and alkali, by many heavy metals (in particular zinc, cadmium, copper and mercury), and by primary alcohols, ketonic reagents and oxidising agents. The stable alkali and alkaline earth salts of penicillin were extremely easily soluble in water, and no organic cation forming a relatively insoluble salt with penicillin suitable for purification purposes was found.

The selection for the methods of purification of penicillin was therefore limited to distribution between different solvents and water, and to adsorption methods. Chromatographic methods have been used extensively. In combination with a reduction process with aluminium-amalgam it has been possible by these methods to produce penicillin preparations from which crystalline salts could be made. The purest material obtained at Oxford has an activity of about 1,000 Oxford units per mg., and is capable of inhibiting the growth of certain bacteria at a dilution of about 1 : 50,000,000.

### Bacteriostatic Properties, Pharmacology, Chemotherapeutic Action in Animals

Once a protein-free preparation containing a stable salt of penicillin was obtained it became possible to examine in detail its biological properties.

For the first biological experiments very crude preparations were used. Their antibacterial properties proved to be the same as those found by Fleming, who used crude penicillin-containing culture fluid. It was noted in addition that *Actinomyces bovis* and the group of anærobic organisms causing gas gangrene were sensitive, but unfortunately not the tubercle bacillus.

So great was the antibacterial power of even the crudest extracts that at that time—not realising the extraordinary potency of penicillin—we believed them to be fairly pure. In actual fact we know now that they contained about 1 % of pure penicillin. However, on the assumption that they were fairly pure, certain biological investigations were undertaken.

It was shown that the extracts were remarkably non-toxic to mice—a 20 g. mouse showed little or no disturbance after injection of 10 mg. of the sodium salt. It has since been found that 20 mg. of a much more highly purified extract can be given without any deleterious effects. Not only were the extracts relatively innocuous to the whole animal, but leucocytes and tissue cultures withstood many hundreds of times the concentration needed to inhibit such organisms as the streptococcus. In the light of present knowledge of the gross impurity of the original extracts, one can only be thankful that the mass of impurities, as well as the penicillin, were so little toxic.

Penicillin was readily absorbed in animals after intramuscular or subcutaneous injection, and from the small intestine. It could not of course be given by mouth because the acid of the gastric juice destroyed it, nor by rectum as the bacteria present there inactivated it. It was largely excreted, still in an active form, in the urine of the mouse, rabbit and cat, and to a certain extent in the bile and saliva, though not in the tears or pancreatic juice of the cat. Though penicillin was readily soluble and diffusible, it did not pass in detectable quantities from the blood into the cerebro-spinal fluid.

In agreement with Fleming's observations it was found that the action of penicillin was bacteriostatic, in that it merely inhibited the growth of organisms and did not kill them quickly, as did poisonous antiseptics such as proflavine. The respiration of bacteria, as measured in a Warburg-Barcroft apparatus, was not affected by quite strong solutions, in contrast to most antiseptics which, acting on some protoplasmic constituent, rapidly cause the cessation of respiration. This bacteriostatic effect was reflected in the morphological changes undergone by sensitive bacteria when grown in a dilution of penicillin not sufficient to cause complete inhibition of growth. By interference with division, giant forms were produced.

Most antibacterial substances such as ordinary antiseptics and the sulphonamides are, for one reason or another, not active in the presence of pus, and hence their therapeutic efficacy is severely limited. It was therefore a particularly fortunate property of penicillin that pus, tissue autolysates, blood and serum had no inhibitory effect on its activity. It was found too that the number of organisms present had little effect on its inhibitory power—again a contrast with the sulphonamides.

In view of this combination of great antibacterial power with low toxicity, it was not altogether surprising that " mouse protection tests " gave a clear demonstration of the chemotherapeutic properties of penicillin. With appropriate dosage almost complete protection was afforded to batches of mice infected intraperitoneally with lethal doses of streptococci and staphylococci and intramuscularly with *Cl. septique*.

### Early Observations on Human Subjects

It will be seen from this account that a fairly complete knowledge of its properties, both chemical and biological, had been obtained before penicillin was used on man. In terms of the labour involved it was, however, a big step from experiments on mice to making observations on the human subject, for the mould produces very little of the active substance. Months elapsed before enough material could be accumulated to try the first injection on man.

Injection in the human subject disclosed that some substance was present in the crude penicillin preparations which caused a rigor and sharp rise of temperature. This had not been suspected from observations in animals. By good fortune the pyrogenic effect was due not to the penicillin but to an impurity which could be removed.

Insufficient material had been accumulated for the first 2 cases treated, and although both patients, who were seriously ill, did well for a time, they relapsed and further treatment could not be carried out for lack of material.

In the course of some months enough was accumulated (partly prepared in Oxford and partly in the laboratories of *Imperial Chemical Industries*) to treat by parenteral injection a further 18 patients. During the course of these observations it became clear that the behaviour of penicillin in man was no different from its behaviour in mice and cats. Toxic reactions, apart from the pyrogen, were not observed and some striking recoveries of patients infected with staphylococci were obtained. Suitable dosage was worked out and the principles of treatment were formulated. At the same time, penicillin was shown to be valuable for local application in various septic conditions.

### Industrial Production and Chemistry

Considerable interest was aroused both in Britain and in the U.S.A. by the demonstration of the chemotherapeutic possibilities of penicillin in naturally occurring disease in man. All the main facts have now been amply confirmed and commercial firms and others are continually improving and developing methods of very large scale production by mould fermentation. One of the chief obstacles to be overcome in large-scale work is the destruction of penicillin which is brought about by the enzymes of many air bacteria. The most careful precautions against contamination are necessary at all stages of growth of the mould.

Immediately after the chemotherapeutic properties of penicillin were established, work on the elucidation of its chemical constitution was actively pursued at Oxford, in the *Sir William Dunn* School of Pathology by Dr. E. P. Abraham and one of us (E. C.) in collaboration with Dr. Wilson Baker and Sir Robert Robinson in the *Dyson Perrins* Laboratory. Subsequently many other chemists both in England and America have started work on the penicillin problem. It is not possible at present to describe in detail the chemical work carried out, but it can be stated that it has led to the elucida-

tion of the chemical constitution of the breakdown products of penicillin and has opened the way to eventual synthesis.

While no doubt large quantities of penicillin will be produced by culture of the mould, there is little prospect that sufficient for general use will be available for some time to come. It is probable that, if synthesis can be achieved, a new range of chemotherapeutic agents will be produced with properties varying in different ways from those of the original substance.

Now that it appears probable that penicillin will be used on a very large scale it is interesting to look back on the early days when we were many times assured that it was too unstable ever to be a practical proposition, and that in any case the vast amount of culture medium needed to produce small quantities of penicillin was an almost insuperable bar to its production.

We should like to emphasise that the work, covering as it did a wide field of investigation, could not have been carried through without the close collaboration of the various workers whose names appear on the papers concerned with penicillin which have come from this laboratory. The clinical work, too, was made possible only by the help and co-operation of many surgeons, physicians and bacteriologists.


REFERENCES

[1] Clutterbuck, P. W., Lovell, R. & Raistrick, H. (1932) *Biochem. J.* **26**, 1907
Dubos, R. J. (1939) *J. exp. Med.* **70**, 1 & 11
Emmerich, R. & Loew, O. (1899) *Z. Hyg. InfektKr.* **31**, 1

[1] [*see BMB* **206**]

Epstein, L. A. & Chain, E. (1940) *Brit. J. exp. Path.* **21**, 339
[2] Fleming, A. (1932) *J. Path. Bact.* **35**, 831
Pasteur, L. & Joubert, J. F. (1877) *C. R. Acad. Sci.* **85**, 101
Roberts, E. A. H. (1937) *Quart. J. exp. Physiol.* **27**, 89

[2] [*see BMB* **205**]




# PENICILLIN FOR SELECTIVE CULTURE AND FOR DEMONSTRATING BACTERIAL INHIBITIONS


ALEXANDER FLEMING, M.B., F.R.C.S., F.R.S.

*from the Inoculation Department, St. Mary's Hospital, London*


My first (1929) paper on penicillin was entitled "On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of *B. influenzae*." The sub-title was inserted because this, the isolation of *B. influenzae*, was the first practical application of penicillin.

In an article in 1932 I mentioned several uses of penicillin:

i. For the isolation of insensitive bacteria from the midst of large numbers of sensitive organisms,
ii. for the demonstration of some bacterial inhibitions,
iii. for the treatment of infections by sensitive organisms.

The third use, undoubtedly its most important one, is dealt with elsewhere, but the first two can be briefly described.

### Isolation of Insensitive Organisms

The human pathogenic organisms may be classified as follows:

| *Penicillin-sensitive* | *Penicillin-insensitive* |
|---|---|
| Staphylococcus | Typhoid, Paratyphoid, Dysentery, Coli group |
| *Streptococcus pyogenes* | *V. cholerae* |
| Other hæmolytic streptococci (other than Group D) | Friedländer's bacillus |
| *Streptococcus viridans* (most) | *B. pyocyaneus* |
| Non-hæmolytic streptococci (most) | *B. proteus* |
| Pneumococcus | Hæmophilic bacilli |
| Gonococcus | Brucella |
| Meningococcus | *B. pestis* |
| *M. catarrhalis* | Enterococcus |
| Diphtheria bacillus | Non-pathogenic gram-negative cocci of the respiratory tract |
| Diphtheroid bacillus (most) | Tubercle bacillus |
| Anthrax bacillus | Yeasts |
| Actinomyces | Moulds |
| *B. welchii* and other clostridia | |

A consideration of this table shows that it is in the respiratory tract that a differential medium containing penicillin is likely to be most valuable in clinical bacteriology. Here the staphylococci, streptococci, pneumococci, and diphtheroid bacilli are sensitive. These are the most commonly found bacteria, and if they are inhibited we are left with little else than Pfeiffer's bacillus, *B. pertussis*, Friedländer's bacillus, and a few non-pathogenic gram-negative cocci, and their isolation becomes easy.

I have already stated that the first practical application in 1929 of penicillin was the isolation of Pfeiffer's bacillus, and we have used it ever since for this purpose.

In 1930 Maclean and myself were able to show that hæmophilic bacteria were present normally in all human mouths and throats. In my original (1929) paper, also, I suggested its use for the isolation of *B. pertussis*, and it has since been used with great success for this purpose by Maclean (1937) and others. This may prove to be its most important application in diagnostic bacteriology, as it is becoming more and more evident that the early bacteriological diagnosis of whooping cough is of the greatest importance in the control of the disease.

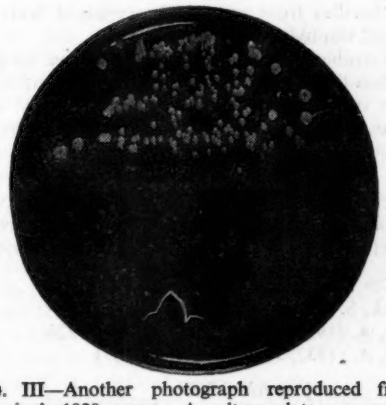


FIG. III—Another photograph reproduced from Fleming's 1929 paper. A culture plate was evenly inoculated with a mixture of staphylococci and *H. influenzae*. Six drops of the crude penicillin-containing solution were then spread over the lower half of the plate, with the result that staphylococcal growth was completely inhibited and a pure culture of *H. influenzae* was obtained. In the upper (untreated) half of the plate, there is a mixed growth of both organisms.

The behaviour of the gram-negative cocci towards penicillin is interesting. The pathogenic types, gonococcus, meningococcus, and *M. catarrhalis*, are among the most sensitive of bacteria, but the various non-pathogenic types normally found in the human mouth and throat are insensitive. Here, then, we have a simple test for the pathogenic types of these cocci which should have a useful application.

In other regions, too, a penicillin medium may be used for special purposes, and by its use in cultures from the puerperal vagina I was able to isolate hæmophilic bacilli in 2 out of 12 cases. A penicillin medium may be used in septic wounds to isolate various bacilli from among the staphylococci, and streptococci, which might obscure them, and I have sometimes found Pfeiffer's bacillus in septic

wounds by this method. On the other hand it is of little use in intestinal cultures, as here the most prominent organisms, and those which are most likely to obscure the others, are *B. coli* and its allies, which are all insensitive to penicillin.

I showed (1932), however, that potassium tellurite had a specific inhibitory action on bacteria which was almost the opposite of penicillin, so that a penicillin-sensitive organism was almost always tellurite-resistant and *vice versa*. Notable exceptions among human pathogenic organisms were the gonococcus, which was sensitive to both, and the enterococcus which was insensitive to both.

Thus by adding an inhibitory quantity of tellurite the penicillin-insensitive organisms were inhibited, and then by adding graded amounts of penicillin many interesting cultural results could be obtained.

Two methods were used for making differential cultures.

i. The infected material was spread on a plate containing a suitable culture medium and then 5 or 6 drops of penicillin (the solution used contained about 5 units per cm.$^3$) were spread over half the plate. The untouched half of the plate therefore gave an ordinary mixed culture, while the penicillin-covered half grew only the penicillin-insensitive organisms. By reason of its simplicity, and of its giving complete and selective cultures on the same culture plate, this method is the most suitable for ordinary clinical purposes, and I have used it with great advantage ever since I introduced it in 1929.

ii. Penicillin may be incorporated in the culture medium in a strength of 0·5 to 1 unit per cm.$^3$ In or on this medium the penicillin-sensitive microbes will not grow, and there remains a culture of only those organisms which are insensitive.

By carefully grading the strength of the penicillin, selective cultures can be made even among the penicillin-sensitive organisms. Thus Craddock (1942) showed that, by adding penicillin to glucose broth in a concentration twice that which would inhibit staphylococci, he was able to isolate the acne bacillus from pus which contained both the acne bacillus and staphylococcus.

The clostridia are not all equally sensitive to penicillin, and it seems likely that, by a careful adjustment of the concentration, one type may be inhibited while another will grow freely, thus aiding in the separation of the different types.

### Demonstration of Bacterial Inhibitions

When cultures are made from the post-nasal space in some catarrhal conditions, and one half of the culture plate is treated with penicillin, it is not unusual to find on one side of that portion of the plate which is thickly planted a pure culture of pneumococcus or streptococcus, and a pure culture of Pfeiffer's bacillus on the other side. From such a plate it is quite clear that when a heavy inoculum is made the pneumococci or streptococci completely inhibit the Pfeiffer's bacilli, the presence of which is revealed when the cocci are prevented from growing by penicillin. It is true that, if the Pfeiffer's bacilli are present in sufficient numbers, they will grow and be recognised when the inoculum is so scanty that the individual colonies are sufficiently separated from the pneumo-streptococcal colonies to be outside the range of the inhibitory power, but when penicillin is used the result is dramatic and cannot be overlooked.

Even more striking results can be obtained by growing a colony of the mould (*P. notatum*) on a culture plate at room temperature for, say, three days. During that time it produces penicillin which diffuses into the culture medium for a distance of perhaps two centimetres. Then two highly coloured bacteria, one sensitive to penicillin (*Staphylococcus aureus*) and one insensitive (*B. violaceus*), are mixed in suitable proportions, and are streaked across the plate up to the mould colony. Near the mould colony only the violet coloured organism (*B. violaceus*) will grow, as the staphylococcus is inhibited by penicillin, but beyond the range of the penicillin the growth will be the bright orange colour of *Staphylococcus aureus*, which completely inhibits *B. violaceus*. Thus, by planting a mixture in such conditions, two pure cultures can be obtained in different parts of the streak. This result is even more dramatic if the cultures are made on a white paper disc placed on the surface of the culture medium. The nutrient material diffuses through the paper and allows growth on the surface, and the colours show up beautifully on the white background of the paper. This culture on paper has the additional advantage that it can be removed, sterilised with formalin vapour, and mounted on a card as a permanent specimen.

These are some of the laboratory uses of penicillin. With its more extensive employment, other useful applications will doubtless appear.

Even in the present days of penicillin shortage, the minute amount necessary for these laboratory uses can always be obtained. The crude culture fluid on which *Penicillium notatum* has grown is amply strong enough, and this can be produced with ease in any laboratory. Special culture media are unnecessary, as the ordinary nutrient broth of the bacteriologist will serve. Nor is an incubator a necessity, for the mould grows and produces penicillin at room temperature only a little more slowly than it does at its optimum temperature of 24° C.


REFERENCES

[1] Craddock, S. (1942) *Lancet*, **1**, 558
[2] Fleming, A. (1929) *Brit. J. exp. Path.* **10**, 226
[3] Fleming, A. (1932) *J. Path. Bact.* **35**, 831
[4] Fleming, A., & Maclean, I. H. (1930) *Brit. J. exp. Path.* **11**, 127
[5] Maclean, I. H. (1937) *J. Path. Bact.* **45**, 472


[1] [*see BMB* **208**] [2] [*see BMB* **203**] [3] [*see BMB* **205**]
[4] [*see BMB* **204**] [5] [*see BMB* **207**]



# OTHER ANTIBACTERIAL SUBSTANCES FROM BACTERIA AND MOULDS


E. CHAIN, Ph.D.

*from the Sir William Dunn School of Pathology, Oxford*


The list below contains a selection of those antibacterial substances from bacteria and moulds which have been examined in some detail. A review of the early work on bacterial antagonism and antibiotics, up to 1941, has been published [29] and a review covering the more recent work is in the press [4].

| *Species* | *Antibiotic* | *Reference* |
|---|---|---|
| **Antibiotics Produced by Bacteria** | | |
| *Ps. pyocyanea* | pyocyanine | 16, 17, 26 |
| ,, | α-oxy-phenazine | 26 |
| ,, | lytic agent | 26 |
| *B. brevis* | gramicidin | 15, 10 |
| ,, | tyrocidine | 10 |
| **Antibiotics Produced by Actinomycetes** | | |
| *A. antibioticus* | actinomycin A. | 31, 32 |
| ,, | actinomycin B. | 32 |
| *A. lavendulae* | streptothricin | 28, 33 |
| *Proactinomyces* Gardner | proactinomycin | 12 |
| **Antibiotics Produced by Penicillia** | | |
| *P. notatum* | penicillin | |
| *P. claviforme*, *P. patulum* | claviformin (also described as patulin) | 2, 5, 6, 9, 37 |
| *P. puberulum*, *P. cyclopium* | penicillic acid | 1, 21 |

**Antibiotics Produced by Penicillia**

| | | |
|---|---|---|
| *P. (Gliocladium) fimbriatum* | gliotoxin | 11, 34, 35 |
| *P. citrinum* | citrinin | 25 |

**Antibiotics Produced by Aspergilli**

| | | |
|---|---|---|
| *A. flavus* | aspergillic acid | 18, 19, 36 |
| " | flavicin | 3 |
| *A. fumigatus* | fumigacin | 30 |
| " | fumigatin | 20 |
| *A. fumigatus mut. Helvola* Yuill | helvolic acid | 7, 37 |
| *A. clavatus* | clavacin | 30 |
| *A. giganteus* | gigantic acid | 22 |

Two antibiotics produced by aspergilli, flavicin and gigantic acid, are very similar to penicillin in their chemical and biological properties. Thus it is evident that the production of penicillin-like substances is not confined to the species *Penicillium notatum* or even to the genus *Penicillium*.

All the other antibiotics, without exception, are less active against bacteria and more toxic to animal tissues than penicillin. With the exception of proactinomycin, helvolic acid and possibly streptothricin they are all protoplasmic poisons, exerting their antibacterial action by combining with a protoplasmic constituent common to all cells (proteins, lipids, *etc.*). However, the crude mixture of gramicidin and tyrocidine, designated as tyrothricin, has been used for the local treatment of infected wounds [15, 23, 24]. None of the other antibiotics has yet received such clinical trial.

Patulin, an antibiotic recently isoiated from culture filtrates of *Penicillium patulum* [2] is identical with the previously described antibiotic claviformin [6, 9]. Like all ordinary antiseptics this substance is a general protoplasmic poison and is toxic to mice and leucocytes. Patulin has been stated by one group of workers to be effective in combating the common cold [2], but another group of workers has not been able to confirm this claim [27].

The least toxic antibiotic, apart from penicillin, appears to be helvolic acid. This substance has many attractive properties from the chemotherapeutic point of view. It is very stable, and a bacteriostatic concentration can readily be maintained in the tissues. When it is given repeatedly, however, the liver suffers severe damage. When more facts about its chemical constitution become known it may be possible to reduce the toxic effect on the liver by modification of the molecule.

Though most of the antibiotics listed above have been obtained in the pure, crystalline state, the chemical constitution of many of them has not yet been elucidated. Several, *e.g.* gliotoxin (a sulphur-containing substance), proactinomycin (an alkaloid-like base), streptothricin and helvolic acid, as well as penicillin itself, are of chemical types hitherto unknown among antibacterial substances. The study of their structure provides many interesting problems and may lead to the synthesis of novel types of antiseptics and chemotherapeutic drugs.

Some antibiotics have in addition great biochemical interest. Thus gramicidin and tyrocidine belong to the very small group of crystalline polypeptides, and, as they are of relatively low molecular weight, the complete elucidation of their constitution may be possible [13, 14] with recently developed methods. This may throw some light on one of the most fundamental and difficult problems in biochemistry, the structure of proteins.

The antibacterial substance notatin [8] (also described as penicillin B or penatin), which is produced by *Penicillium notatum*, differs in nature from the antibiotics, being a glucose dehydrogenase which exerts its antibacterial action through the hydrogen peroxide which it forms. It has no action in the absence of glucose, nor in the presence of catalase, a constituent of all tissue cells. It is extremely toxic to animals and is unlikely to be of use in medicine.


REFERENCES

[1] Alsberg, C. L., & Black, C. F. (1913) *U.S. Dep. Agric., Bur. Plant Ind., Bull.* no. 270
[2] Birkinshaw, J. H., Bracken, A., Greenwood, M., Gye, W. E., Hopkins, W. A., Michael, S. E., & Raistrick, H. (1943) *Lancet*, **2**, 625
[3] Bush, M. T., & Goth, A. (1943) *J. Pharm. exp. Therap.* **78**, 164
[4] Chain, E., & Florey, H. W. (1944) *Ann. Rep. Chem. Soc.* in press
[5] Chain, E., Florey, H. W., & Jennings, M. A. (1942) *Brit. J. exp. Path.* **23**, 202
[6] Chain, E., Florey, H. W., & Jennings, M. A. (1944) *Lancet*, **1**, 112
[7] Chain, E., Florey, H. W., Jennings, M. A., & Williams, T. I. (1943) *Brit. J. exp. Path.* **24**, 108
[8] Coulthard, C. E., Michaelis, R., Short, W. R., Sykes, G., Skrimshire, G. E. M., Standfast, A. F. B., Birkinshaw, J. H., & Raistrick, H. (1942) *Nature*, **150**, 634
[9] Crowfoot, D., & Low, B. (1944) *Lancet*, **1**, 113
[10] Dubos, R. J., & Hotchkiss, R. D. (1942) *Trans. Coll. Phys. Philadelphia*, **10**, 11
[11] Dutcher, J. D. (1941) *J. Bact.* **42**, 815
[12] Gardner, A. D., & Chain, E. (1942) *Brit. J. exp. Path.* **23**, 123
[13] Gordon, A. H., Martin, A. J. P., & Synge, R. L. M. (1943a) *Biochem. J.* **37**, 86
[14] Gordon, A. H., Martin, A. J. P., & Synge, R. L. M. (1943b) *Biochem. J.* **37**, 313
[15] Herrell, W. E., & Heilman, D. (1941) *J. Clin. Invest.* **20**, 583
[16] Hettche, H. O. (1933) *Klin. Wschr.* **12**, 1804
[17] Hettche, H. O. (1934) *Z. ImmunForsch.* **83**, 499
[18] Jones, H., Rake, G., & Hamre, D. M. (1943) *J. Bact.* **45**, 461
[19] Menzel, A. E. O., Wintersteiner, O., & Rake, G. (1943) *J. Bact.* **46**, 109
[20] Oxford, A. E., & Raistrick, H. (1942) *Chem. Ind.* **61**, 128
[21] Oxford, A. E., Raistrick, H., & Smith, G. (1942) *Chem. Ind.* **61**, 22
[22] Philpot, F. J. (1943) *Nature*, **152**, 725
[23] Rammelkamp, C. H., & Keefer, C. S. (1941) *J. clin. Invest.* **20**, 433
[24] Rammelkamp, C. H. (1942) *War Medicine*, **2**, 830
[25] Raistrick, H., & Smith, G. (1941) *Chem. Ind.* **60**, 828
[26] Schoental, R. (1941) *Brit. J. exp. Path.* **22**, 137
[27] Stuart-Harris, C. H., Francis, A. E., & Stansfeld, J. M. (1943) *Lancet*, **2**, 684
[28] Waksman, S. A. (1943) *J. Bact.* **46**, 299
[29] Waksman, S. A. (1941) *Bact. Rev.* **5**, 231
[30] Waksman, S. A., Horning, E. S., & Spencer, E. L. (1943) *J. Bact.* **45**, 233
[31] Waksman, S. A., & Tishler, M. (1942) *J. biol. Chem.* **142**, 519
[32] Waksman, S. A., & Woodruff, H. B. (1941) *J. Bact.* **42**, 231
[33] Waksman, S. A., & Woodruff, H. B. (1942) *Proc. Soc. exp. Biol.* **49**, 207
[34] Weindling, R., & Emerson, O. H. (1936) *Phytopath.* **26**, 1068
[35] Weindling, R., & Emerson, O. H. (1936) *Phytopath.* **27**, 1175
[36] White, E. C., & Hill, J. H. (1943) *J. Bact.* **45**, 433
[37] Wilkins, W. H., & Harris, G. C. M. (1942) *Brit. J. exp. Path.* **23**, 166




# CLINICAL USES OF PENICILLIN


M. E. FLOREY, M.B., B.S.

*from the Central Middlesex County Hospital*


The successful application of experimental laboratory work to therapeutic measures could hardly be better exemplified than in the case of penicillin. Before any trial was made on man, almost the full range of infections capable of response to the drug was known. Equally important was the knowledge that the upper limit of dosage was not likely to be influenced by any toxic effect. Among the other inferences of practical importance that could be made from the laboratory work were : that oral administration of the unprotected drug would be ineffective because of its inactivation

by the acid gastric juice; that frequent administration would be necessary because of the rapid concentration and excretion of penicillin by the kidneys; that it would be unwise to prepare wounds for local application by cleaning with a number of the common antiseptics because of the inactivation of penicillin by heavy metals and by oxidation. The lack of any inhibitory effect on leucocytic activity by therapeutic concentrations of penicillin indicated that the disappearance of pus from lesions infected only by sensitive organisms would probably be a fair criterion of the elimination of sepsis.

With these facts already known, the main points still to be elucidated when clinical trials were begun were: adequate dosage, frequency and routes of administration and the most suitable methods for local application.

### Clinical Trials

The earliest clinical trials, which included some 200 cases, were instigated by the *Sir William Dunn* School of Pathology, Oxford (Abraham, Chain, Fletcher, Florey, Gardner, Heatley & Jennings, 1941; Florey & Florey, 1943). They demonstrated the therapeutic effectiveness of the drug by both systemic and local administration. The cases chosen for treatment were pyogenic infections falling into the following categories:

- Generalised infections accompanied by local lesions in bones, lungs and heart valves
- Acute mastoiditis
- Infections of the skin
- Infections of the conjunctiva, cornea and lacrymal sac
- Chronic blepharitis
- Chronic sinus infections
- Empyema
- Synovitis
- A miscellaneous collection of infective conditions

The causal organism in the majority was the staphylococcus, in others it was the streptococcus or a combined infection by both organisms. A few infections were pneumococcal, gonococcal or due to actinomyces, and occasionally no organism sensitive to penicillin could be isolated.

The results of these early trials at Oxford may be summed up as:

| | |
|---|---|
| Complete recovery | 143 patients |
| Improvement | 43 „ |
| Little or no response | 14 „ |
| *Total* | 200 |

These results appear striking enough to merit some consideration of the criteria adopted in ascribing recovery specifically to penicillin treatment. The first criterion was bacteriological. Pyogenic organisms present in lesions became scanty during treatment and eventually disappeared. If, concurrently with their disappearance, the local and general signs of inflammation subsided, function returned, and healing began, these effects were considered as fairly attributable to removal of infection by penicillin. The fact that most patients treated in the first year or two of clinical trials were considered moribund, had not responded to any other treatment, or suffered from an infection which had persisted for many months or years without improvement, gave additional support to the conclusion that penicillin was the instrument whereby recovery took place.

About 150 cases of burns were also treated at about this time by Dr. Colebrook working for the *Medical Research Council* in the Burns Unit at Glasgow (Clark, Colebrook, Gibson, Thomson & Foster, 1943), and Flight Lieutenant Bodenham (Bodenham, 1943). No staphylococcal or streptococcal infection of a burn failed to disappear under adequate treatment. Healing was accelerated or successful skin grafts were carried out following the removal of the organisms from the surface of the burn.

Work in the U.S.A. has confirmed and amplified the British results mainly in systemic treatment (Keefer, Blake, Marshall, Lockwood & Wood, 1943). Of 500 cases reported, including some where the infection was not the only pathological condition (some cases were also suffering from cancer, aplastic anæmia, aneurism, etc.) the results were:

| | |
|---|---|
| Recovery or improvement | 366 |
| No response | 40 |
| Deaths | 94 |
| *Total* | 500 |

This series included 129 cases of gonorrhœa, of which the treatment was uniformly successful and subsequent reports (*Lancet*, 1943; Garrod, 1943) have confirmed the very rapid response to treatment in this condition irrespective of whether the infection is sulphonamide-resistant or not. Pneumonias were also treated with success, but the results recorded were by no means so striking as in gonococcal infections.

In neither the British nor the American series did recovery occur in subacute bacterial endocarditis (*Streptococcus viridans* infection) even after prolonged treatment.

In England, following the earliest trials, it was thought advisable to carry out a comparative trial of acute infections from which patients would in any event recover. By comparing penicillin-treated cases with similar acute infections treated by current methods the effects which might be attributable to the drug could be gauged.

Infections of the hands were chosen for this trial (Florey, M. E., & Williams, 1944). A hundred "control" patients were treated by recognised methods and a second 100 were treated in every way similarly except that penicillin was applied locally from operation onwards in place of (usually) hypochlorite preparations and (when considered necessary) sulphonamides by mouth. Bi-weekly bacteriological examinations showed that, in 75% of control wounds, pyogenic organisms persisted from the first incision until complete healing. The remaining 25% lost their infection or showed a diminished growth only when healing was far advanced. In 95% of the penicillin-treated cases, these organisms had markedly diminished in numbers or had disappeared before the end of the first week.

From the clinical point of view this rapid elimination of sepsis resulted in the following marked differences:

| *Control* | *Penicillin-Treated* |
|---|---|
| Continuance of pain and throbbing for variable periods following incision | Almost invariable relief of pain from incision and first application of penicillin onwards |
| Production of pus until healing was well advanced | Pus scanty or absent following operation |
| Formation of much granulation tissue with consequent contraction, limitation of movement, and sometimes painful scars | Little formation of granulation tissue. Rapid and more complete restoration of movement and smooth and painless scars |
| In severe infections, usually much sloughing, death of tendons and bone, and loss of digits | No loss of digits or any other tissue when treatment was begun at first operation |

The retention of digits and restoration of function are particularly valuable assets in an industrial community where early return to work depends on full use of the hands. The working time saved to the 100 penicillin-treated cases totalled in the aggregate a number of years.

A further series of cases has been treated in the war zone (*Lancet*, 1943; Garrod, 1943). Here local application of the drug has enabled the early suturing of war wounds of the soft tissues to be carried out with almost uniform success. In no case did deleterious results follow this unorthodox treatment, although in some wounds complete union was not achieved and a few broke down. Results at 3 weeks were:

| | |
|---|---|
| Complete union | 104 |
| Subtotal union | 60 |
| Failure | 7 |
| *Total* | 171 |

In wounds complicated by compound fractures systemic as well as local treatment was employed, but the results were not so strikingly successful as in the soft tissue injuries. Results are improving, however, with developments in technique and the earlier administration of penicillin.

Gas gangrene was treated in 7 patients. The systemic route was used. Four of these patients recovered, but further improvements in results may well follow adaptations in the method of administration.

Of the penetrating brain wounds treated, some had an established infection, while others were treated within a few days, at a stage when infection had hardly developed. Results were satisfactory and healing occurred without fungus formation in all but one case. Three out of the 23 patients died, however, but in two of these the infection by gram-positive

organisms had been controlled and the third was inadequately treated.

This series of war injuries demonstrated well the successful and rapid results obtained when penicillin treatment is begun in the early stages of infection, rather than when the condition is so well established as to be less accessible either to local or systemic exhibition of the drug.

Investigations up to date have therefore shown the value of penicillin:

i. As a preventive of infection in wounds, enabling a potentially septic wound to be treated in much the same way as an aseptic one,

ii. in the promotion of healing in burns and for ensuring the success of skin grafts,

iii. in infections (due to sensitive organisms) either (*a*) chronic, or (*b*) of such severity as to render the prospect of death likely, which have not responded to other forms of treatment,

iv. in acute infections due to sensitive organisms,

v. in the rapid curing of gonorrhœa including sulphonamide-resistant cases,

vi. in pneumonia,

vii. probably in gas gangrene, but here numbers have been few and methods not fully tried out.

### Special Considerations

*a. Excretion.* Very early in the Oxford clinical trials the rapid excretion of penicillin by the kidneys was seen to be both a problem and an asset. In the days when every unit was precious, efforts were made to recover the drug from the patient's urine, but the labour and inconvenience were great and were justified only by the scarcity of material. The rapid deterioration of the penicillin when acted on by bacteria in the urine also made the yield very variable. One patient was able to pass urine every hour for 11 hours after a single dose. These specimens were assayed immediately and, although he was still excreting penicillin when the last specimen was taken, more than 75 % of the dose was regained. Such was not the case when urine was collected in larger amounts and at longer intervals. Rammelkamp & Keefer (1943b), who have carried out detailed work on absorption, excretion and toxicity of penicillin, have also obtained variable yields from the urine, but it is not yet clearly established whether the total amount is excreted or whether part is destroyed in the body.

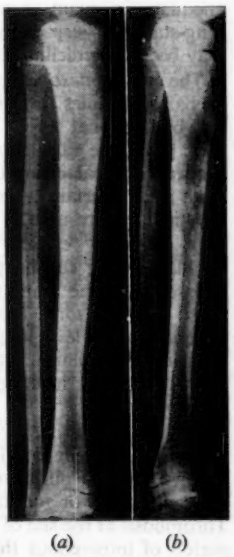
Its rapid elimination has been of value in determining the time taken for penicillin injected by various routes to reach the circulation and for a single dose to disappear. After intramuscular or intravenous injection, penicillin may be detected in the urine almost immediately, and after intrapleural injection within an hour. Penicillin is also concentrated by the kidneys, as after a single small dose it can be detected in the urine for well over 24 hours, even when the concentration has at no time been great enough to be detected in the blood by present methods. Administration for urinary infection has therefore been successful when repeated only once or twice in 24 hours, and staphylococcal and streptococcal urinary infections have been cleared up by doses which were relatively very small.

*b. Effect on blood cells.* On all cases treated from Oxford systemically and on many treated locally regular blood examinations were carried out. In no case was there a fall in the erythrocyte count during penicillin treatment, and in all severely ill cases, except one puerperal patient in whom a profuse lochia continued throughout treatment, there was an average rise of 250,000 to 500,000 erythrocytes per week. In view of the poor condition of the myocardium in septic patients, there seems therefore to be little indication for adding the strain of blood transfusion to the work of the heart. The leucocyte count appeared to give a good indication of the progress of the treatment. If it was low from toxæmia (or possibly from previous sulphonamide administration) at the beginning, it began to rise within 24 hours. If high in response to infection it dropped steadily as the infection was controlled, but never below normal limits. The leucocyte count should therefore come into prominence as an indication of progress, in treatment by penicillin, where the temperature chart often does not reflect improvement in the early stages.

*c. Accessibility of thecal and serous cavities.* Passage of penicillin between the blood stream and serous cavities seems to be slight (Rammelkamp & Keefer, 1943a, 1943b; Fleming, 1943; Florey, M. E. & Florey, H. W., 1943), and for treatment of infections of the meninges, pleural cavity, joints and peritoneum local rather than intramuscular or intravenous injection is therefore indicated. The physiological basis of the apparent (relative) impermeability of these membranes to penicillin still remains to be elucidated.

*d. Toxicity.* Any signs of toxicity have been carefully sought by observation of the patient, by regular examinations of blood urea and urine, and by hæmatological examinations. Occasionally the blood urea has risen but it has fallen again on the day on which penicillin has been discontinued. No case in which albuminuria has developed has been recorded in the English series, but when present initially it has cleared up under penicillin therapy. Fever often increases when penicillin is first administered, but gradually subsides again. This rise of temperature may be due to a more rapid absorption of bacterial degradation products rather than to any toxic effect of the drug.

*e. Radiological findings.* Special mention should be made of the radiological appearances of infected bone treated with penicillin. A very rapid rarefaction of the affected bone occurs, and radiography in less than a week, often in 2 or 3 days, suggests deterioration, whereas the clinical signs indicate that the septic process is under control. Further radiographs demonstrate the rapid reformation of bony tissue as compared with control cases, in which (in the series of hand infections) sequestrum formation occurred more often than not. In acute infections no sequestrum formation has been found in the English penicillin-treated series. If, in a chronic infection, dead bone has already separated before the beginning of penicillin treatment, rarefaction will occur around the sequestrum and will make its presence and site more obvious to the surgeon.



(*a*) (*b*)

FIG. IV—A case of osteomyelitis with pyæmia in a male child of 8. The child received just under 2,000,000 units of penicillin intramuscularly.

(*a*) Right tibia and fibula before penicillin treatment was begun.

(*b*) Three weeks later, at the conclusion of penicillin treatment.

The characteristic rarefaction which occurs in infected bone during penicillin treatment is well illustrated. The boy, who had been desperately ill, made a good recovery without surgical intervention.

Photograph reproduced from the *Lancet*.

*f. Bacteriological desiderata.* Mention of the organisms sensitive to penicillin has been made elsewhere (Fleming, 1929; Abraham *et al.*, 1941). It is clearly futile to attempt penicillin treatment when the organism concerned is insensitive. Emphasis has already been laid on the speed and completeness with which organisms usually disappear during treatment with penicillin. A few further points, however, should be borne in mind. Bacteria do not readily disappear from lesions if there is any dead tissue present such as a slough or sequestrum, nor do they disappear when access by the drug is not complete. This may occur with systemic treatment in undrained abscess cavities or in serous cavities, where the penicillin may be brought by the blood stream only as far as the periphery of the infected area. It may also occur with local treatment in sinuses which have not been completely opened up, or in septic areas where the incision has not reached the whole site of infection. Disappearance and elimination of infection, therefore, depend on (*a*) a sensitive organism, (*b*) adequate dosage, (*c*) absence of dead tissue, (*d*) full access to the site of infection. It has not been uncommon to find coliform organisms appearing or increasing in number during penicillin administration, but they do not appear to affect the clinical course or delay healing materially, even when green or pinkish "gram-negative pus" is present.

*In vitro*, bacteria readily become penicillin-resistant, and some clinical cases have been reported where resistance of the order of 4 to 8 times developed during treatment although clinical improvement continued (Florey & Florey, 1943; Rammelkamp & Maxon, 1942). Sulphonamide-resistant strains have been found, however, to be fully sensitive to penicillin.

### Methods of Treatment

*a. Systemic administration.* In the early clinical trials the greatest difficulty encountered was to find the adequate dosage. The ring test (Abraham *et al*, 1941) was not sufficiently sensitive to demonstrate any bacteriostatic effect in the blood after doses which were believed on theoretical grounds to be large enough to produce bacteriostasis throughout the body. Eventually, after trial and error, a baby provided the foundation on which an adequate dose to eliminate infection was established as 1,000 units per pound [454 g.] of body weight in 24 hours. Later, the more delicate slide-cell technique was adopted, in which the inhibition of bacterial growth by the blood serum could be accurately followed. This served to establish that intravenous or intramuscular injection should be repeated not less than 3-hourly, the standard single dose for an adult being 15,000 Oxford units. Later work by Rammelkamp & Keefer (1943b) has shown that even when nearly 3 times the dose is given there is no prolongation of the time during which the drug can be detected in the blood stream. Good effects have been produced in acute infective conditions by much smaller doses than 15,000 units in the Mayo Clinic (Herrell, Heilman & Williams, 1942; Herrell, 1943) and elsewhere, but although these results are very satisfactory it would seem wise for general use to recommend a dosage which has been fonud adequate in many conditions—acute, well-established, or long-standing—especially in view of the fact that organisms may become penicillin-resistant.

The *gastro-intestinal* route was tried—by mouth in specially prepared capsules to resist solution by the acid gastric juice, and by duodenal tube. Although some absorption certainly took place, the variable rate at which the drug was absorbed rendered the bacteriostatic concentration in the blood inconstant and uncertain when doses of a size which is at present practicable were used.

In the early clinical work, *subcutaneous* injection was avoided owing to the pain associated with it. Although according to the work of Rammelkamp & Keefer (1943b) there may be some advantage in using this route, as an adequate blood level is apparently maintained for longer than after intramuscular or intravenous injection, the large volume they used was inconvenient and may explain the slow absorption.

For the best results by *intravenous* use it is essential to use a pyrogen-free preparation (the pyrogenic impurity can be removed during purification). The penicillin may be given intermittently, or continuously by a "drip" infusion. Thrombosis at the site of injection is common after a variable period of intravenous therapy, although with good samples of the drug the "drip" can often be continued for some days.

For this and other reasons the *intramuscular* route was adopted for general use in the British work. Providing a good technique is used, most samples cause little general or local reaction, but some samples have been encountered which do cause prolonged pain after intramuscular injection. There can be little doubt that this reaction, the thrombosis after intravenous injection, and other reactions of a minor character are due to impurities. As the penicillin used therapeutically is still far from pure, usually containing only 10–20 % of the pure substance, it is to be hoped that the quality of penicillin produced for clinical purposes will improve. By the use of the intramuscular route, material which causes a sharp rise of temperature on intravenous administration can be employed without causing a pyrexial reaction.

*b. Local administration* has been used extensively in Britain, largely because of the scarcity of supplies, but many instances might be given where it has advantages over systemic treatment. Where an infection is definitely localised, and there is a surface or cavity which will retain a preparation of penicillin, a much greater concentration can be applied to the site of infection and, by using a suitable vehicle, the action of a single dose may be prolonged for as long as 24 hours. For this purpose the following preparations have been found useful:

i. A dry calcium-penicillin powder of low potency (i.e. a relatively unpurified preparation containing a low percentage of pure penicillin).

ii. A powder composed of a calcium-penicillin preparation diluted with sulphanilamide or sulphathiazole powder to a strength of 2,000 to 5,000 units of penicillin per gram. Such a powder is very suitable for insufflation.

iii. Solutions in distilled water of 250 to 1,000 units per cm.$^3$

iv. A paste made up of lanette wax SX, oil (*e.g.* castor or arachis) and water to which penicillin is added to a strength of 150 to 250 units per cm.$^3$ With this preparation the activity of the drug may be retained for at least 24 hours.

v. Mixed with vaseline or some other base as an eye ointment—500–800 units per cm.$^3$

Various methods of local application have been used, such as simple dressings impregnated with the paste preparation, insufflation of powder, or spreading of the powders over infected surfaces. A method which, when applicable, is very effective because it embodies the principle of maintaining a closed cavity, is to aspirate pus and inject a solution by syringe and needle. An alternative method is to remove the purulent and damaged tissue from an abscess cavity or wound, suture the raw edges and insert one or more narrow rubber tubes (retaining them by a suture) leading down to the depths of the cavity. In the latter method, the cavity is aspirated and the penicillin solution is injected through the tubes by means of a syringe. One extremity of the tube projects through the dressings. Injections are made 6- to 12-hourly according to the acuteness of the condition, for a minimum period of 5 days.

Sinuses of many years' duration have been treated successfully by injection of the solution under pressure, thereby opening up their many ramifications. The mouth of the sinus is closed with a sterilised rubber bung in order to retain the fluid from one injection until the next. This retention of the drug continuously *in situ* over a period of time has been a cardinal feature of treatment. Because of its bacteriostatic rather than bactericidal action, time must be allowed for the inhibited bacteria to perish by phagocytosis or otherwise. Any temporary cessation of the inhibitory influence of penicillin may permit further bacterial proliferation and so prolong the time necessary to eliminate infection.

The duration of treatment has depended on the time taken to eliminate bacteria from lesions. There is no indication that increased dosage beyond the point necessary to secure complete bacteriostasis is of any value. The effect of higher dosage is merely to cause more rapid excretion of the drug by the kidneys. Bearing in mind the possibility that the pathogenic organisms may develop resistance to penicillin, a fully bacteriostatic dose should be given from the beginning. There has been little evidence, except in subacute bacterial endocarditis, that an infection by a sensitive organism will not respond to prolonged treatment. The point to be remembered is that treatment must be continued until all foci of infection have been removed. As mentioned earlier, when dead tissue is present, surgical removal may be necessary, but radiological and bacteriological findings are better indications for this than is the temperature chart.

### Assessment of Progress

*a. General infections.* When treating a case with penicillin, assessment of progress is liable to be based on the signs familiar in sulphonamide therapy. This may, unfortunately, lead to erroneous conclusions. The temperature, for instance, in a case of osteomyelitis may assume a swinging character when penicillin is first given, and it never falls rapidly, as often occurs with the sulphonamides. Often the temperature mounts to a higher level than before treatment was instituted.

By the time the temperature has become normal, which may take from 2 to 3 weeks, resolution has largely taken place. The radiograph, which in infections of bone shows rapid and often startling rarefaction, should be regarded as evidence of the fast absorption of damaged bony tissue, and not of deterioration in the condition.

The earliest signs of progress are noticed by the nursing staff. Within a day or two the patient sleeps better, eats better and is relieved of much pain. Bacteriological examina-

tion carried out twice a week will reveal steady diminution and eventually total disappearance of the infecting organism, provided that the drug has full access, locally or systemically, to the whole of the infected part, and that there is no dead tissue remaining to form a nidus for the infection.

If there is no diminution of bacterial growth within a week, consideration must be given to the advisability of aspiration or surgical interference in order to gain better access to localised lesions.

The blood count is also of value in assessing the arrest of infection. A steady fall of the leucocyte count to within normal limits and a rise in the erythrocyte count are indications that good progress is being made.

*b. Local infections.* In local lesions the subsidence of pain, redness, swelling and induration, the disappearance of pus, and the return of function, are all indications of progress. It must always be remembered that, apart from the relief of pain following incision and the local application of penicillin, there is little that is dramatic in the course of recovery from the infection. Treatment may be stopped within 5 days in acute conditions, but should often be continued for as long as 3 to 4 weeks in well established infections where there is much dead tissue to be absorbed. The final result will depend in such cases on continuing the treatment with penicillin until the bacteriological evidence indicates that infection has been arrested.


REFERENCES

[1] Abraham, E. P., Chain, E., Fletcher, C. M., Florey, H. W., Gardner, A. D., Heatley, N. G., & Jennings, M. A. (1941) *Lancet*, **2**, 177

[2] Bodenham, D. C. (1943) *Lancet*, **2**, 725

[3] Clark, A. M., Colebrook, L., Gibson, T., Thomson, M. L., & Foster, A. (1943) *Lancet*, **1**, 605

[4] Fleming, A. (1929) *Brit. J. exp. Path.* **10**, 226

[5] Fleming, A. (1943) *Lancet*, **2**, 434

[6] Florey, M. E., & Florey, H. W. (1943) *Lancet*, **1**, 387

Florey, M. E. (1943) *unpublished data*

Florey, M. E., & Williams, R. (1944) *Lancet*, **1**, 73

[7] Garrod, L. P. (1943) *Brit. med. J.* **2**, 755

[7] *Lancet* (1943) **2**, 742



[1] [*see BMB* **212**] [2] [*see BMB* **238**] [3] [*see BMB* **234**] [4] [*see BMB* **203**]
[5] [*see BMB* **236**] [6] [*see BMB* **233**] [7] [*see BMB* **237**]

[*All other references are to be found among those given in BMB* **242**]


# REVIEW OF SELECTED PAPERS

## *Discovery : Early Investigations : Bacteriological Applications*

*In this section are described the first investigations on penicillin, and the early and later application of its selective bacteriostatic properties in bacteriological techniques. The work which led to the effective application of penicillin in the treatment of bacterial infections is described in another section.*

**203**

**ON THE ANTIBACTERIAL ACTION OF CULTURES OF A PENICILLIUM WITH SPECIAL REFERENCE TO THEIR USE IN THE ISOLATION OF *B. INFLUENZAE***
by A. Fleming, *British Journal of Experimental Pathology*, **10**, 226–236, June 1929

In this paper, published in 1929, Professor Alexander Fleming, of *St. Mary's* Hospital, London, first described his discovery of penicillin and the investigations by which he established certain important facts about the substance.

" While working with staphylococcus variants a number of culture-plates were set aside on the laboratory bench and examined from time to time. In the examinations these plates were necessarily exposed to the air and they became contaminated with various micro-organisms. It was noticed that around a large colony of a contaminating mould the staphylococcus colonies became transparent and were obviously undergoing lysis."

It was evident that a substance antagonistic to the bacteria had been formed by the mould and had diffused into the surrounding agar. He therefore subcultured the mould for further study and found that broth in which it had grown for a week or two acquired marked inhibitory properties to many common bacteria.

*Characteristics of the mould.* On a solid medium [composition not stated] a colony of the mould, in Professor Fleming's own words, " appears as a white fluffy mass which rapidly increases in size and after a few days sporulates, the centre becoming dark green and later in old cultures . . . almost black. In 4 or 5 days a bright yellow colour is produced which diffuses into the medium. In certain conditions a reddish colour can be observed in the growth. In broth the mould grows on the surface as a white fluffy growth changing in a few days to a dark green felted mass. The broth becomes bright yellow." The reaction of the medium became alkaline, *p*H 8·5 to 9. Growth was slow at 37° C., and most rapid at about 20° C. No growth took place anærobically. Other moulds of several species were tested, including 8 other strains of penicillium, but only one, which was apparently identical with the original strain, produced any inhibitory substance. The active strain resembled *Penicillium rubrum* [but was later identified as *Penicillium notatum* (Fleming, 1932).]

*Tests of activity.* The antibacterial activity of the broth was tested in two ways : (i) A strip was cut out of an agar plate and the resulting gutter was filled with agar mixed with an equal amount of the broth. The bacteria to be tested were streaked across from the gutter to the edge of the plate. The active substance diffused from the gutter into the surrounding agar, where it inhibited the growth of susceptible organisms. (ii) Serial dilutions of the active broth were made in plain broth and the tubes were inoculated with equal amounts of bacterial culture. The staphylococcus was found to be a suitable organism for testing the activity of the broth, as it was very sensitive. It was not necessary to filter the broth before testing, as no appreciable growth of the mould took place in 24 hours at 37° C.

*Development of the active substance.* When 200 cm.³ of nutrient broth in a 500 cm.³ Erlenmeyer flask was inoculated with mould spores and incubated at 20° C. it gradually developed antibacterial activity until at 7 or 8 days it would inhibit the growth of staphylococci when diluted 1 : 500 or 1 : 800. On keeping longer, the activity diminished. The droplets of fluid which formed on the surface of the mould had a higher titre than the broth, sometimes as great as 1 : 20,000.

The filtered broth lost activity in a few days, but was more stable if the *p*H was adjusted to 6·8. Heating for an hour at 56° or 80° C. and boiling for a few minutes did not destroy the activity, but boiling for an hour reduced it and autoclaving for 20 minutes at 115° C. abolished it. Boiling was less destructive if the broth was first neutralised. The active substance was soluble in water and saline and was not removed by Seitz filtration.

*Antibacterial tests.* In a long series of dilution tests, it was shown that some species of bacteria were inhibited by the broth in dilutions up to 1 : 800, while others, relatively insensitive, were not inhibited by a dilution of 1 : 5. Staphylococcus (*aureus* and *epidermitis*), streptococcus and pneumococcus were the most sensitive and *B. coli*, *B. typhosus*, *Ps. pyocyanea*, *B. proteus*, *V. cholerae* and *Strept. faecalis* the

least. *B. diphtheriae* and *B. anthracis* occupied an intermediate position. Different strains of *Strept. viridans* varied widely in sensitivity. *B. pseudotuberculosis rodentium*, *B. pullorum* and *B. dysenteriae* were as insensitive as the colityphoid group. These results were confirmed by the gutter test, by which also the meningococcus and gonococcus were shown to be as sensitive as the staphylococcus, and *H. influenzae* to be insensitive. The original mould was not inhibited by the broth. It was shown that staphylococci were not suddenly killed but disappeared gradually over some hours, when in contact with penicillin.

*Toxicity.* The active broth was shown to be no more toxic than plain broth when given intravenously to rabbits and mice, and to cause no irritation or other undesirable effect when applied to the conjunctiva and to large ulcerated areas in man. A concentration which completely inhibited the growth of staphylococci did not disturb the normal function of leucocytes more than plain broth.

*Use in bacteriology.* It was shown in various ways that penicillin broth suppressed the growth of staphylococci and streptococci on an agar plate, while allowing the development of insensitive organisms from a mixed culture. For the isolation of *H. influenzae* (Pfeiffer) its use was particularly recommended. The penicillin broth could be incorporated in the agar or, more simply, it could be spread over half the plate after inoculation. Boiled blood agar was recommended as a medium. In 25 clinical cases of " influenza " the bacillus grew from the throat swab of 23 with the aid of penicillin broth and from only 8 without the penicillin.

In his discussion the author points out that his penicillin broth inhibited sensitive organisms at a higher dilution than carbolic acid or other chemical antiseptics in common use. It was in addition non-irritant and non-toxic. Experiments to investigate its value in treating pyogenic infections were in progress. In addition to its possible clinical use, it was certainly useful in bacteriology, notably in facilitating the isolation of *H. influenzae.*


REFERENCE

[1] Fleming, A. (1932) *J. Path. Bact.* **35**, 831


[1] [*see BMB* **205**]

**204**

## ON THE OCCURRENCE OF INFLUENZA BACILLI IN THE MOUTHS OF NORMAL PEOPLE

by A. Fleming & I. H. Maclean, *British Journal of Experimental Pathology*, **11**, 127–134, April 1930

In the work here reported Professor Fleming and Dr. Maclean, of the Inoculation Department, *St. Mary's* Hospital, London, investigated the mouths of normal people for the presence of hæmoglobinophilic bacilli (*H. influenzae* (Pfeiffer) and related organisms). Previous workers had reported finding such organisms in between 25 % and 80 % of normal subjects. In the present work they were found in all of 36 mouths, and usually in several situations, *i.e.* gum, tonsil and post-nasal space. The strains isolated were very variable as regards their cultural characters. In 6 mouths studied with especial care both influenza and para-influenza strains were isolated from the gums in every case, and in the rest para-influenza bacilli were always found in the gums. The success of their work was attributed by the authors to the use of penicillin for differential culture, since in a suitable concentration it suppressed the growth of the gram-positive cocci and *M. catarrhalis*, while allowing the growth of the influenza group of bacilli.

The filtrate from a culture of *Penicillium notatum* could be used in three ways :

i. The infected material was spread on an agar plate and then half the plate was spread with 4 to 8 drops of penicillin broth. This simple method produced a plate, half of which showed a complete culture and half a differential culture.

ii. A more exact method was to make several dilutions of the penicillin broth in a suitable fluid medium and inoculate all the tubes with infected material. The influenza bacillus was at least 40 times less sensitive than the staphylococcus, so that if, for example, the staphylococcus was inhibited at 1 : 400, suitable dilutions for isolating influenza bacilli would be 1 : 3, 1 : 6, and 1 : 10. After 24 or 48 hours' incubation the cultures were plated out.

iii. Plates were made with a solid medium in which penicillin broth was incorporated in known dilutions.

These methods enabled the authors to isolate influenza bacilli when, on ordinary culture, they would have been overgrown or inhibited by other organisms.

**205**

## ON THE SPECIFIC ANTIBACTERIAL PROPERTIES OF PENICILLIN AND POTASSIUM TELLURITE

by A. Fleming, *Journal of Pathology and Bacteriology*, **35**, 831–842, November 1932

In this paper the author recalls the discovery of penicillin (Fleming, 1929) and describes its use in bacteriology. He also reports on its first use as a dressing on septic wounds.

When his strain of *Penicillium notatum* was grown in trypsin digest broth for 6 or 7 days at 20° C. the filtered broth completely inhibited the growth of staphylococci when diluted 1 : 800. The broth, which was alkaline, sometimes preserved its potency for as much as 3 months, if neutralised to about *p*H 6·8 and subsequently adjusted when necessary.

By incorporating this penicillin broth in culture media, bacteria could be divided into two broad groups: (*a*) those whose growth was prevented by concentrations of 1 % or less, and (*b*) those which would grow even in the presence of a 5 or 10 % concentration. The methods of using the penicillin broth in culture media were the same as those described by Fleming & Maclean (1930).

After describing the use of potassium tellurite in bacteriology (dilutions of 1 : 1,000 spread on a plate, or 1 : 20,000 incorporated in the medium where suitable), the author gives a table showing penicillin and tellurite to be complementary, the organisms sensitive to one being insensitive to the other and *vice versa*. Exceptions were the enterococci, moulds and yeasts, and some types of *B. coli* and *B. proteus*, which were sensitive to neither, and the gonococcus which was sensitive to both.

*Examples of the Use of Penicillin*

i. To isolate the hæmophilic bacteria, which are penicillin-insensitive, from mixed cultures [*see* Fleming & Maclean, 1930].

ii. To isolate the acne bacillus, which is only moderately sensitive, from material in which it is mixed with the staphylococcus, which is highly sensitive [*see* Craddock, 1942].

iii. To distinguish the saprophytic gram-negative cocci (such as *M. flavus*), which are insensitive, from the pathogenic gram-negative cocci which are highly sensitive.

iv. To demonstrate the inhibition of one bacterial species by another. For example, a plate was sown with a mixed culture of a streptococcus and *V. cholerae*, and penicillin was spread over half the plate. In the presence of penicillin the streptococcus did not grow, and a pure culture of *V. cholerae* was found. On the other half of the plate the streptococcus suppressed the growth of the vibrio and was itself found in pure culture. Many similar examples are given.

v. In combination with potassium tellurite, to isolate one species of bacterium from a mixture. For example, sputum was cultured in broth containing a high concentration of penicillin and a low concentration of tellurite. The penicillin inhibited the growth of the gram-positive cocci, while the tellurite inhibited para-influenza bacilli and other gram-negative organisms. *H. influenzae*, which is slightly less sensitive to tellurite than the para-influenza organisms, grew out in pure culture.

vi. As a dressing for septic wounds. The penicillin-containing broth, which appeared perfectly harmless, had been used to dress a number of indolent septic wounds, and had " certainly appeared to be superior to dressings containing potent chemicals." The author believed that it did not act by killing the bacteria directly. Its use as a dressing presented certain difficulties, because of the labour of preparation and the instability of the active principle.


REFERENCES

[1] Craddock, S. (1942) *Lancet*, **1**, 558
[2] Fleming, A. (1929) *Brit. J. exp. Path.* **10**, 226
[3] Fleming, A., & Maclean, I. H. (1930) *Brit. J. exp. Path.* **11**, 127


[1] [*see BMB* **208**] [2] [*see BMB* **203**] [3] [*see BMB* **204**]

206

### THE FORMATION FROM GLUCOSE BY MEMBERS OF THE *PENICILLIUM CHRYSOGENUM* SERIES OF A PIGMENT, AN ALKALI-SOLUBLE PROTEIN AND PENICILLIN—THE ANTIBACTERIAL SUBSTANCE OF FLEMING

by P. W. Clutterbuck, R. Lovell & H. Raistrick, *Biochemical Journal*, **26**, 1907–1918, 1932

Professor Raistrick and his co-workers at the *London School of Hygiene & Tropical Medicine*, University of London, followed up Fleming's original discovery by initiating chemical investigations on penicillin. In this paper they describe the production of penicillin by Fleming's mould in a synthetic medium, and preliminary attempts to isolate the antibacterial substance. [A yellow pigment, chrysogenin, and an alkali-soluble protein were also found in the metabolism solution, but these will not be discussed here.]

Fleming's mould was reported by Dr. Charles Thom to be closely related to *P. notatum* Westling in the *P. chrysogenum* Thom series. The organism was grown in conical flasks on a modified Czapek-Dox medium of the following composition:

| | |
|---|---|
| $NaNO_3$ | 3·0 g. |
| $KH_2PO_4$ | 1·0 g. |
| KCl | 0·5 g. |
| $MgSO_4,7H_2O$ | 0·5 g. |
| $FeSO_4,7H_2O$ | 0·01 g. |
| Glucose | 40·0 g. |
| Distilled water to | 1 litre |

The temperature of incubation was 25° C. At the end of a period of incubation the metabolism solution was sterilised by passing it through a Seitz filter. Its antibacterial power was then determined by inoculating one loopful of the test organism into tubes containing varying dilutions of the filtrate in trypsinised horse-muscle broth. After incubation at 37° C. for 24 hours, opacity readings were taken.

The antibacterial substance was found to reach its maximum concentration at about the 16th to 20th day, when the metabolism fluid inhibited the growth of *Strept. pneumoniae* at a dilution of 1 : 1,280. Further incubation resulted in a loss of activity.

Penicillin was found to be quickly inactivated by evaporation *in vacuo* at 40° C. in both acid and alkaline solutions, but it was moderately stable at *p*H 3·6, which caused the flocculation of most of the protein and chrysogenin. The filtrate was then adjusted to *p*H 5·5 and concentrated *in vacuo*. This resulted in a partial loss of activity. At *p*H 2 (but not at *p*H 7·2) the active substance could be extracted by ether. Evaporation of the ethereal extract to dryness gave an inactive residue, but evaporation *in vacuo* over water left an aqueous solution which, after adjusting to *p*H 7·2, still contained some of the original activity.

207

### A MODIFICATION OF THE COUGH PLATE METHOD OF DIAGNOSIS IN WHOOPING COUGH

by I. H. Maclean, *Journal of Pathology and Bacteriology*, **45**, 472–473, September 1937

The author recommends the use of penicillin as an aid to isolating *H. pertussis*. A few drops of broth from a culture of *Penicillium notatum* were spread over one-half of 50 "cough plates"; 47 were positive on the penicillin side and only 33 on the untreated side. Swabs taken from the pharynx just after coughing and planted on similar plates gave 75–80% positive results with penicillin and about 10% without. By both methods the use of penicillin allowed a heavier inoculum, as the number of colonies of streptococci and staphylococci was greatly reduced.

208

### USE OF PENICILLIN IN CULTIVATION OF THE ACNE BACILLUS

by S. Craddock, *Lancet*, **1**, 558–559, 9/5/42

The author, working in the Inoculation Department of *St. Mary's* Hospital, states that in acne lesions the acne bacillus is usually accompanied by *Staph. albus*, but that by the use of penicillin pure cultures could be obtained.

*Method.* A tube of glucose broth (*p*H 6·8) was boiled to expel dissolved gases. When cool, pus from an acne lesion was inoculated into it. The filtrate from a culture of *Penicillium notatum* was added, diluted so that the broth finally contained a concentration of penicillin twice as great as that needed to prevent the growth of staphylococci (this concentration was determined by a preliminary titration). Hot sterile "vaseline" [petroleum jelly] was run on to the surface to a depth of 0·5–1·0 cm., and the tube was incubated at 37° C. After about 60 hours a growth of the bacillus was visible and after 4 days it was abundant.

By this method the bacillus was isolated in pure culture from all of 47 specimens of material from acne pustules, comedones and sebum, though without penicillin 34 grew staphylococci in addition.

## *Chemotherapeutic Action: Pharmacology: Bacteriological Investigations*

*Under this heading is described the first successful use of penicillin as a chemotherapeutic drug in experimental animal infections, which was made possible by the extraction of active, although still impure, material from the crude penicillin-containing fluid. Preliminary observations on the pharmacology of the drug were made. This work provided evidence that penicillin might have important therapeutic possibilities. A bacterial enzyme which destroys penicillin, and the morphological effects of penicillin on bacteria, are also reported.*

209

### PENICILLIN AS A CHEMOTHERAPEUTIC AGENT

by E. Chain, H. W. Florey, A. D. Gardner, N. G. Heatley, M. A. Jennings, J. Orr-Ewing & A. G. Sanders, *Lancet*, **2**, 226–228, 24/8/40

In this, the first paper on penicillin by the Oxford workers, Professor Florey and his collaborators describe how, following up some work on lysozyme, "it occurred to two of them [Dr. Chain and Professor Florey] that it would be profitable to conduct a systematic investigation of the chemical and biological properties of the antibacterial substances produced by bacteria and moulds." One such substance was penicillin, which had been discovered, named and described by Fleming in 1929. It was produced by a mould of the species *Penicillium notatum* and had promising antibacterial properties.

Methods of culture, assay and extraction of penicillin were not described in this paper, the authors confining themselves to reporting their first antibacterial, toxicity and therapeutic tests. The tests were carried out with crude penicillin in the form of a brown powder, freely soluble in water, prepared from the culture medium. [It is now known that this powder contained only from 1% to 2% of pure penicillin.]

*Antibacterial action in vitro.* A number of pathogenic bacteria were tested and found to be inhibited by dilutions of one in several hundred thousand, *i.e. Cl. welchii, Cl. septique, Cl. oedematiens, C. diphtheriae, Strept. pyogenes, Strept. viridans, Strept. pneumoniae* and staphylococcus. Penicillin appeared not to be bactericidal, but to interfere with multiplication of the bacteria.

*Toxicity.* Ten mg. intravenously had no apparent ill effect on a mouse weighing 23 g. Larger doses, 10 mg. 3-hourly for 56 hours, given subcutaneously to two rats, had no general effect, though there appeared to be a transient decrease in the polymorph count and some histological evidence of damage to the renal tubules. In a cat, 40 mg. intravenously (representing a blood concentration of 1 : 5,000) did not alter the blood pressure, heart beat or respiration. An isolated cat's heart slowed during the perfusion of 1 : 5,000 and 1 : 10,000 solutions, but recovered as soon as perfusion with penicillin was stopped. Human leucocytes remained active in a 1 : 1,000 solution.

*Absorption and excretion.* Penicillin in solution was absorbed from the intestine of the rat without damage to the mucosa, and from the subcutaneous tissues, and was excreted by the kidneys. It was present in the blood and was excreted in the urine, which it coloured bright yellow.

*Therapeutic effect.* Five therapeutic experiments were performed, two with *Strept. pyogenes*, two with *Staph. aureus* and one with *Cl. septique*. The first two were injected intraperitoneally and the last, as a spore suspension with 2·5% calcium chloride, intramuscularly. Penicillin was given by subcutaneous injection, 3-hourly for the first 3 days and then at longer intervals. In the first experiment with streptococci, where treatment was only continued for 12 hours, life was prolonged, but at 10 days 25 out of 50 treated mice were dead, compared with 21 out of 25 controls. In the next experiment a smaller total dose was given—7·5 instead of 10 mg.—but it was spread over 45 hours; 24 out of 25 treated mice survived, whereas all the controls (25) were dead in 16 hours. A similar result was obtained with *Staph. aureus*. In the first experiment treatment was inadequate, though continued for 55 hours, but when it was continued for 4 days, 21 out of 24 mice survived, whereas all the controls were dead in 48 hours. The *Cl. septique* infection killed the 25 controls in 17 hours, but after treatment, which lasted 10 days, 24 out of 25 mice receiving a total of 38 mg., and 18 out of 25 receiving half that dose, survived. It was noticed that the mice infected with streptococci and staphylococci looked gravely ill for the first few hours, even when undergoing treatment, but thereafter recovered and were normal in 36 to 48 hours.

**210**

**AN ENZYME FROM BACTERIA ABLE TO DESTROY PENICILLIN**

by E. P. Abraham & E. Chain, *Nature*, **146**, 837, 28/12/40

Though *B. coli* was insensitive to the antibacterial power of penicillin, no inactivator of penicillin was found in the culture fluid. The possibility remained that the bacterial bodies themselves contained an inactivating substance. A suspension of the bacilli was ground up in a bacterial crushing mill and the resulting bacterial extract was found to inactivate penicillin completely. The inhibitory substance had the properties of an enzyme, and the authors named it "penicillinase." The inactivation took place with equal readiness under aerobic or anaerobic conditions; there was no uptake of oxygen or appearance of acid groups during the reaction.

The enzyme was not found in *Staph. aureus* but was present in *M. lysodeikticus* which was sensitive to penicillin, though less so than the staphylococcus. It was absent from yeast and from *Penicillium notatum*. An air bacterium, a gram-positive rod, also contained the enzyme, and in this case it was present also in the culture fluid.

**211**

**MORPHOLOGICAL EFFECTS OF PENICILLIN ON BACTERIA**

by A. D. Gardner, *Nature*, **146**, 837–838, 28/12/40

The author, Professor in Bacteriology in the University of Oxford, reports that penicillin altered the morphology of bacteria when present in concentrations too small to inhibit growth completely. The effect could be seen at concentrations down to a 10th, and in some cases even a 30th, of that needed for complete inhibition.

The principal changes appeared to be due to incomplete division, giving rise to swollen and grotesque giant forms and, in the case of bacilli and vibrios, to long filaments. Such forms would presumably be abnormally vulnerable to leucocytes and other defence mechanisms, and penicillin might therefore be expected to have an effect *in vivo* at concentrations too low for complete bacteriostasis.

## *First Clinical Trials: Production: Biological Properties*

***In the single comprehensive paper reviewed under this heading is reported the successful application, in the treatment of human infections in a small number of cases, of the data obtained by laboratory experiments. Large-scale laboratory production was described, but material was still very scarce. The work reported in the previous year was considerably amplified. Steps towards the purification of penicillin were made.***

**212**

**FURTHER OBSERVATIONS ON PENICILLIN**

by E. P. Abraham, E. Chain, C. M. Fletcher, H. W. Florey, A. D. Gardner, N. G. Heatley & M. A. Jennings, *Lancet*, **2**, 177–188, 16/8/41

This paper embodies the full account of the first investigations by the Oxford workers. It includes details of the growth of *Penicillium notatum* and laboratory large-scale production; the methods of assay of the active substance; chemical methods for extraction and purification of penicillin; a fuller investigation of its bacteriostatic action and toxicity, and of absorption and excretion; and finally a report of the first therapeutic trials on man.

**[It should be noted that the penicillin preparations used in the work here described contained varying amounts of the pure substance and never more than 5%. The paper is therefore more valuable for its description of the properties of penicillin than for the quantitative data it contains.]**

*Growth of the Mould*

For the production of penicillin, the mould was grown on the modified Czapek-Dox medium of Clutterbuck *et al.* (1932), with tap water instead of distilled water; 10% of yeast extract was added, or 2% if the medium was being placed under mould already grown. [Other media producing a higher yield of penicillin are now in use, but the formulae are as yet unpublished.]

When a spore suspension of *Penicillium notatum* was sown into this medium and incubated at 24° C., the mycelium developed, remaining submerged for 3 days and then reaching the surface (the depth of the fluid being 1 cm.), where it formed a continuous mat. If the medium was disturbed even by gentle rocking, formation of the mat was delayed. By the 6th or 7th day the mat was corrugated and covered by bluish-green spores, sometimes with yellow droplets lying on the surface.

The *p*H of the medium fell until the 3rd day and then rose to over *p*H 8. Penicillin production was maximal at about *p*H 7. Various conditions affecting growth were noted, and the need for strict sterility was emphasized, as certain bacteria destroyed penicillin. For maximum production the depth of the medium did not exceed 1·5 to 2 cm.

*Method of Assay*

A method was devised by which the antibacterial activity of solutions could be rapidly tested. Glass or vitreous porcelain cylinders 9·6 mm. long and of 5·1 mm. internal diameter, at the lower end internally bevelled and ground flat, are placed on the surface of an agar plate which has been uniformly sown with a broth culture of *Staph. aureus* and dried. The ground end makes a water- and bacteria-tight junction with the agar, so that non-sterile fluids can be placed in the cylinder for testing without contaminating the agar plate. [It has since been found advantageous to heat the cylinder for a moment before placing it on the agar.] The active substance diffuses from the fluid into the agar and inhibits the growth of the staphylococcus round the cylinder for a distance proportional to its concentration in the fluid. Several samples can be tested on one plate.

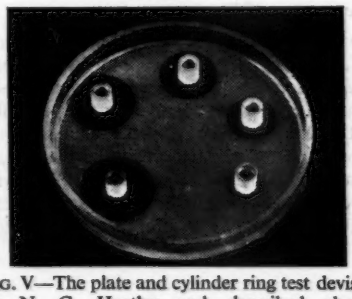


FIG. V—The plate and cylinder ring test devised by N. G. Heatley and described above.

Reproduced by permission of *Endeavour*.

Various points affecting the use of the cylinders are discussed. Tests done in triplicate with solutions so diluted as to produce a ring of inhibition not more than 25 mm. in diameter have probably an error of not more than ± 25% and possibly much less. A standard penicillin solution should be included on every plate for comparison.

*Laboratory Large-Scale Production*

The mould was grown in closed rectangular porcelain vessels, 27·5 × 22 × 6 cm. external diameter, with a side arm for filling and inoculation. The medium, enough to fill the vessels to a depth of 1·7 cm., was sterilized in the vessels, inoculated with a few drops of spore suspension and incubated at 24° C. An apparatus for withdrawing and replacing the medium under the mat of mould is described in detail.

*Extraction from the Medium*

Some details of the method of extraction are given, which rest upon the following general principle. At *p*H 2 the active substance will pass from aqueous solution into ether, amyl acetate, and certain other organic solvents. As, however, in aqueous solution it is rapidly destroyed at this *p*H at room temperature, the extraction must be carried out rapidly or in the cold. It passes back into aqueous solution at *p*H 6–7. The substance obtained as a sodium salt preparation after a single extraction of this type was found to be pyrogenic for rabbits and man. For further purification it was passed in ethereal solution through a chromatographic adsorption column of Brockmann's alumina. The final product, the sodium salt in aqueous solution, was a deep reddish-orange, yellow when diluted, and had a faint characteristic smell and a bitter taste. It was suitable unmodified for therapeutic injection, after blowing off the ether, or it could be dried, yielding a voluminous hygroscopic yellow powder, and redissolved when required. [Extraction based on these principles is still in general use for industrial production.]

*Antibacterial Action*

The purest preparation available at the time of writing inhibited several species of bacteria, including *Staph. aureus* at a dilution of 1 : 1,000,000 and the gonococcus at 1 : 2,000,000. The work of Fleming (1929) on the susceptibility of different bacterial species was, in general, confirmed. In addition, the clostridia were found to be highly sensitive, *S. gaertneri* and *S. typhi* to be of intermediate sensitivity, and *Myc. tuberculosis* to be insensitive.

A staphylococcus when repeatedly subcultured in medium containing penicillin became rapidly resistant—eventually 1,000 times less sensitive. It was shown that this acquired resistance was not due to an enzyme which destroyed penicillin.

Penicillin was shown to be, like the sulphonamides, bacteriostatic not bactericidal. It differed in its antibacterial action from the sulphonamides in the following ways:

i. It was active at a much higher dilution.
ii. It was little affected by the number of bacteria present.
iii. It was not inhibited by serum, blood, pus, autolysed tissues or peptones.

*Toxicity to Tissue Cells*

*Human leucocytes* adhering to a coverslip were inverted over a well-slide containing penicillin dissolved in a special physiological saline with 10 % of serum. The leucocytes were watched under the microscope for 4 hours. In 1 : 100 penicillin they died immediately, but in 1 : 500 they were indistinguishable from control preparations. By comparison, proflavine killed immediately at 1 : 20,000, and 2 : 7 diaminoacridine-monohydrochloride and sodium hypochlorite at 1 : 10,000. The sulphonamides were by this test little more toxic than penicillin, as the leucocytes, though they became inactive, survived for 2 hours or more in 1 : 500 sulphanilamide and in saturated solutions of sulphapyridine and sulphathiazole. [Purer preparations of penicillin have since been shown to be less toxic to leucocytes than these (Florey & Jennings, 1942).]

In *tissue culture* it was shown that there was no difference between fibroblasts and epithelial tissues in respect of their sensitivity to penicillin; the smallest concentration at which any toxic effect was seen was about 1 : 5,000. Fibroblasts exposed to a 1 : 1,600 solution for 48 hours began to grow again when the drug was removed, and hen's blood macrophages similarly recovered after 42 hours in a 1 : 200 solution.

The *central-nervous system* was the site chosen for an experiment on local application. A 1 : 1,000 solution of penicillin was made to flow into the cerebrospinal space of two rabbits through a cisternal puncture by slowly injecting 11 cm.³ of 25 % sodium chloride intravenously. In one rabbit 2·0 cm.³ of penicillin solution was drawn in and in the other 1·5 cm.³ Neither showed any disturbance, and histological examination 6 days later was completely negative. Examination of the cortex of other rabbits to which penicillin had been applied directly was also negative.

*Absorption and Excretion*

These were investigated in the cat, the rabbit and man. It was shown in the cat that penicillin was absorbed from the subcutaneous tissues and from the intestine. It could be detected in the blood for at least 1 hour after subcutaneous or intravenous injection, and for at least 3 hours after intestinal administration. It was present in the urine for 6 hours or more and was excreted in high concentration in the bile, where it reached its maximum at 3 or 4 hours. After intravenous injection, the saliva contained a trace, but none could be detected in the tears, the pancreatic juice or the cerebrospinal fluid. About 50 % of the penicillin passed into the urine, whatever the route of administration. In the rabbit, the substance could rarely be detected in the blood but a good concentration was present in the bile. After intravenous injection 20 % to 50 % was excreted in the urine, but less than 20 % after intestinal administration.

In man, penicillin could be detected in the blood up to 3 hours after intravenous injection or administration by duodenal tube, and was excreted in the urine for 6 hours or more. At least 50 % passed into the urine, from which it could be extracted to be used again. Material containing the pyrogenic impurity was freed of the pyrogen by its passage through the body.

The fate of the penicillin not excreted in the urine was not ascertained. There was no appreciable destruction when penicillin was incubated with sterile tissue from a number of organs.

*Clinical Therapeutic Trials*

Clinical trials were begun on the basis of the properties of penicillin established in the laboratory. Its non-toxicity in concentrations far above those which would inhibit bacteria had been confirmed. It was rapidly eliminated by the kidneys, and frequent or continuous administration was therefore necessary to maintain an anti-bacterial concentration continuously in the body. It was known to be absorbed from the intestine, but as it was destroyed by acid it could be given by mouth only if the stomach were alkalinised or the penicillin were enclosed in enteric (acid-resisting) capsules. Fæces destroyed penicillin, probably by bacterial action, so rectal administration was not practicable. It was absorbed from the subcutaneous tissues, but the local effect of injecting concentrated solutions in man was not known.

For these reasons the first human subjects were treated by giving the penicillin intravenously in a "continuous drip" infusion of saline. The dose was injected 2- or 3-hourly into the tubing conveying the saline to the vein or (Case 5) dissolved in the saline and given continuously. Five patients were treated by this method. The first (Case 1) was a febrile and severely emaciated man of 43 with a mixed staphylococcal and streptococcal pyæmia. He was treated for 5 days, after which penicillin supplies were exhausted, but during that time pus formation strikingly diminished and in some sites it ceased altogether and healing started. Appetite and general condition improved and the temperature fell. After 3 days more the patient was afebrile. Equally good results were obtained in a streptococcal wound infection (Case 2) and an acute staphylococcal osteomyelitis with septicæmia (Case 5). A large carbuncle resolved without discharging and without scar formation (Case 3). A boy of 4½ (Case 4), with cavernous sinus thrombosis and lung abscesses due to *Staph. aureus*, became strikingly better during 9 days' treatment with penicillin, the cerebrospinal fluid becoming sterile after 6 days. He was afebrile and appeared on the road to recovery 4 days after the end of treatment when a cerebral vascular accident occurred, due to rupture of a mycotic aneurysm. At *post-mortem* the cavernous sinus region was occupied by granulation tissue, and in the lung, also, healing was in progress. All that remained of the infection was small groups of cocci in microscopic areas of necrosis at the centre of the granulation tissue.

In an infant of 6 months (Case 6) with a persistent urinary infection due to *Staph. aureus*, penicillin was given by mouth with sodium bicarbonate (to neutralise the acid gastric

juice) for 7 days. Though no penicillin could be detected in the blood, the urine became strongly bacteriostatic and the infection disappeared. Three patients with acute conjunctivitis and one with staphylococcal keratitis (Cases 7, 8, 9, 10) were treated by local applications of penicillin. The symptoms were rapidly and strikingly relieved and a quick recovery took place.

During the treatment of these patients there were no toxic symptoms (except for rigors in two cases due to the pyrogenic impurity, which had not been removed). The total leucocyte count usually fell, lymphocytes and polymorphs being equally affected, but the fall ran parallel with the resolution of the infection. The blood urea was slightly above normal in 2 cases, but without any abnormality in the urine.

These results suggested very strongly that penicillin, properly used, would overcome staphylococcal and streptococcal infections in man, though the authors ended by emphasizing that much further investigation would be needed, for which larger supplies of penicillin are essential.


REFERENCES

[1] Clutterbuck, P. W., Lovell, R., & Raistrick, H. (1932) *Biochem. J.* **26**, 1907

[2] Fleming, A. (1929) *Brit. J. exp. Path.* **10**, 226

[3] Florey, H. W. & Jennings, M. A. (1942) *Brit. J. exp. Path.* **23**, 120


[1] [*see BMB* **206**] [2] [*see BMB* **203**] [3] [*see BMB* **213**]

# *Further Chemotherapeutic, Bacteriological, and other Investigations*

*This section includes a wide variety of papers on the biological properties of penicillin and its chemotherapeutic action in experimental infections. Chemical investigations and reports of therapeutic trials in man are described in later sections.*

213

## SOME BIOLOGICAL PROPERTIES OF HIGHLY PURIFIED PENICILLIN

by H. W. Florey & M. A. Jennings, *British Journal of Experimental Pathology*, **23**, 120–123, June 1942

The preparations of penicillin used by Abraham *et al.* (1941) contained 40 to 50 units per mg. [about 4–5 % of pure penicillin]. As soon as preparations containing 250 to 500 units per mg. [25–50 % of pure penicillin] were available, the principal biological tests were repeated on the more active material.

i. *Antibacterial activity.* Growth of the staphylococcus was completely inhibited by a dilution between 1 : 24,000,000 and 1 : 30,000,000 and partially inhibited up to 1 : 160,000,000. The activity towards other organisms was proportionately increased.

ii. *Toxicity to mice.* Mice given 20 mg. intravenously showed no reaction. As 20 mg. of the less pure preparations had caused sickness it was evident that toxicity was diminished with the removal of impurities.

iii. *Toxicity to leucocytes.* Leucocytes survived and moved about sluggishly for an hour in a 1 % solution in spite of its hypertonicity.

*The calcium salt.* In all previous experiments the sodium salt of penicillin had been used. The calcium salt, unlike the sodium salt, was not hygroscopic and was therefore easier to handle. Ten mg. intravenously, however, produced symptoms in a mouse, and 20 mg. subcutaneously produced immediate distress and subsequent sloughing at the site of injection. Though a 1 % solution caused no reaction in a rabbit's ear it was thought that the calcium salt was unsuitable for therapeutic use. [The preparation of calcium salt tested contained 5 % of penicillin. Calcium salt preparations are in practice found satisfactory for local application if suitably diluted.]

*Assay of penicillin.* The authors preferred the plate and cylinder method for routine work, keeping the dilution method for occasionally checking the strength of standard or other preparations. The former method was much quicker and did not require a sterile solution.


REFERENCE

[1] Abraham, E. P., Chain, E., Fletcher, C. M., Florey, H. W., Gardner, A. D., Heatley, N. G., & Jennings, M. A. (1941) *Lancet*, **2**, 177


[1] [*see BMB* **212**]

214

## *IN VITRO* TESTS OF PENICILLIN POTENCY

by A. Fleming, *Lancet*, **1**, 732–733, 20/6/42

In this paper the author surveys the methods available for testing the antibacterial activity of penicillin. The newer antiseptics, of which the sulphonamides and penicillin are the chief examples, differ from the older in two ways: (i) they prevent bacterial growth (bacteriostatic action) instead of killing the bacteria (bactericidal action), and (ii) they act more strongly on some species of bacteria than on others (selective action), instead of acting equally on all species.

With their discovery it became necessary to devise tests which would measure bacteriostatic activity and which did not depend on showing that bacteria had been killed.

*Tests with the growing mould.* The mould is streaked on one side of a culture plate and grown at a suitable temperature (about 20° C. for *Penicillium notatum*). When the growth is well developed, the bacteria to be tested are streaked across the plate at right angles to the mould. Several species of bacteria can be tested on one plate. If the medium used for the mould is unsuitable for the bacteria it can be covered by a thin layer of another medium before the bacteria are planted, as the penicillin diffuses readily from the old into the new layer of agar.

### (*a*) *Qualitative*

*Tests with Fluid Solutions*

i. *The gutter method.* This was described in a previous paper (Fleming, 1929).

ii. *Agar cup method.* A culture plate is inoculated with staphylococcus or some other suitable bacterium. Circular discs are cut out of the agar by a cork borer, and the bottom of the "cup" so formed is sealed with 2 drops of melted agar. The solution to be tested is placed in the cup, whence the active substance diffuses into the surrounding agar. The broth from a satisfactory culture of *Penicillium notatum* inhibits growth for about 15 mm. from the edge of the cup. Graduated dilutions of penicillin produce zones whose width is directly proportional to the concentration of the penicillin. This method, compared with the cylinder method of Abraham *et al.* (1941), requires no special apparatus and is not affected by the presence of erythrocytes which, in the cylinder test, lie on the surface of the agar and prevent diffusion of the active substance.

These two methods are of value as qualitative tests, the former to test a number of bacterial species on one plate, the latter to test a number of samples against one species. The applicability of the test obviously depends on the diffusibility of the active substance in agar; the author suggests that such diffusion may be related to diffusibility in the body and therefore to therapeutic activity.

### (*b*) *Quantitative*

*The titration method.* A flask of broth is inoculated with the test organism, and used for making serial dilutions of the penicillin. A volume of 0·5 or 1·0 cm.³ per tube is as satisfactory as a larger volume. The sensitivity of an organism or the activity of a penicillin solution can be accurately measured by this method.

To standardize the test for titrating penicillin it is advisable for all workers to use the same strain of staphylococcus and a uniform inoculum and culture medium. The medium suggested consists of peptone (Evans) 1g.; NaCl 0·5 g.; glucose 1 g.; Andrade's indicator 1 cm.³; water to 100 cm.³ The result is read at 24 hours, and the end-point is shown by a change of the indicator to red.

### (*c*) *Tests in the Presence of Blood*

Up to 25 cm.³ of blood can be incorporated in an agar plate for the gutter or cup test, or the titration method can be used for any organisms which will grow in blood.

The slide-cell technique is also available and has the advantage that the bacteria which are not inhibited form colonies which can be counted.


REFERENCES

[1] Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., & Florey, H. W. (1941) *Lancet*, **2**, 177

[2] Fleming, A. (1929) *Brit. J. exp. Path.* **10**, 226


[1] [*see BMB* **212**] [2] [*see BMB* **203**]

215

## ZINC PEROXIDE, PROFLAVINE AND PENICILLIN IN EXPERIMENTAL *CL. WELCHII* INFECTIONS

by J. McIntosh & F. R. Selbie, *Lancet*, **2**, 750–752, 26/12/42

The authors, Professor of Pathology and Assistant Pathologist at the *Bland Sutton* Institute of Pathology of the *Middlesex* Hospital, London, designed their experiments to show the effect of a single dose of penicillin given locally at the site of an anærobic infection.

*Cl. welchii* was grown anærobically for 18 hours in 4% *Difco* proteose-peptone in distilled water. An injection containing 2 or 4 × $10^9$ washed bacteria in 0·1 cm.³ with 0·1 cm.³ of 5% calcium chloride was given into the right thigh of each mouse. This was equivalent to at least 100 lethal doses. The infection became generalised in the blood stream within 3 or 4 hours. A dose of 34 Oxford units of penicillin in 0·2 cm.³ was given at the same site. Survival was recorded up to 3 days.

*Results.* All control mice died within 24 hours. In 3 different experiments, a total of 42 mice treated with penicillin at the time of infection all survived. Five out of 6 mice treated at 2 hours survived, but there were no survivals after treatment at 3 or 6 hours, though life was prolonged.

216

## CHEMOTHERAPEUTIC DRUGS IN ANAEROBIC INFECTIONS OF WOUNDS

by J. McIntosh & F. R. Selbie, *Lancet*, **1**, 793–795, 26/6/43

The margin of safety between the toxic dose and the therapeutic dose was found, in mice, to be greater for penicillin than for sulphonamides or acridines. Penicillin had no undesirable local effect.

Therapeutic tests were done on mice by injecting intramuscularly 100 minimal lethal doses of the organisms suspended in 2·5% $CaCl_2$ solution. Thirty-four Oxford units of penicillin were given locally into the same site. The interval between injection and treatment was one hour.

*Results.* Penicillin was greatly superior to sulphathiazole in the treatment of *Cl. welchii* and *Cl. oedematiens* infections, but somewhat less effective than sulphathiazole against *Cl. septicum.* The acridines occupied an intermediate position.

[*A more general summary of this paper, with details of the technical methods used, will be published in a future number of BMB.*]

217

## COMBINED ACTION OF ANTITOXIN AND LOCAL CHEMOTHERAPY ON *CL. WELCHII* INFECTION IN MICE

by J. McIntosh & F. R. Selbie, *Lancet*, **2**, 224–225, 21/8/43

The authors compared the effects of giving antitoxin alone and antitoxin with penicillin to mice infected experimentally with 100 lethal doses of *Cl. welchii* in 2·5% calcium chloride.

When 5 units of antitoxin were given, in one or two doses from 3 to 6 hours after infection, eight out of thirty-six mice survived for 3 days or more. When 50 units of penicillin were given with the antitoxin nineteen out of thirty-six survived. Better results were seen when treatment was started 3 hours after infection than when it was started later. Proflavine was considerably less effective than penicillin as an adjunct to antitoxin.

[*A more general summary of this paper, with details of the technical methods used, will be published in a future number of BMB.*]

218

## LOCAL CHEMOTHERAPY IN EXPERIMENTAL LESIONS OF THE EYE PRODUCED BY *STAPHYLOCOCCUS AUREUS*

by J. M. Robson & G. I. Scott, *Lancet*, **1**, 100–103, 23/1/43

The authors, a pharmacologist and an ophthalmic surgeon working in the Department of Pharmacology of the University of Edinburgh, studied the effect of penicillin (and other antibacterial substances) in experimental lesions of the eye due to the staphylococcus.

*Technique.* A strain of *Staphylococcus aureus* which would produce an acute and severe infection of the eye in rabbits was selected. A 24-hour culture was diluted to contain 1,500 bacteria per cm.³ A hypodermic needle with a blunt bevel was inserted into the cornea and the diluted culture was injected until a small blister was raised under the corneal epithelium.

The severity of the infection varied in different rabbits, but in each rabbit the lesions in the two eyes were remarkably similar. Severe conjunctivitis, corneal ulcers and iritis were always produced, and usually a hypopyon. The final condition varied from slight scarring to gross destruction of the eye.

Penicillin was applied to one eye, and saline solution as a control to the other. Treatment was started 1 hour after the infection and was given every hour for 48 hours and thereafter hourly during the day.

*Result.* Penicillin was applied to one eye in 17 rabbits and, of these eyes, 14 suffered slight or negligible damage. Among the control eyes, the damage was slight in only one and in the rest was classed as moderate or severe. Cultures from the treated eyes were sterile during treatment and for several days after.

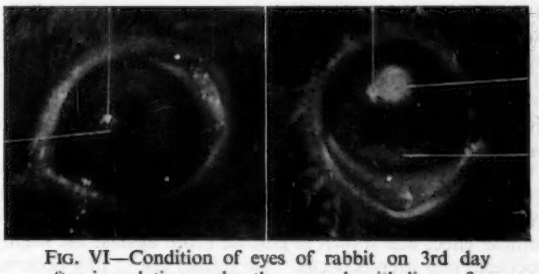


FIG. VI—Condition of eyes of rabbit on 3rd day after inoculation under the corneal epithelium of a staphylococcal culture. In the eye (left side) treated with penicillin, only a faint infiltrate developed, and this later disappeared. In the untreated eye there is considerable corneal ulceration with hypopyon. This photograph is reproduced from the *Lancet*.

When the treatment was started 24 hours after infection it was almost without effect.

[*Reference is made above only to those parts of the original paper in which the use of penicillin is described. A more general abstract of the same paper has previously been published* (*BMB* **53**).]

219

## THE PRODUCTION AND TREATMENT OF EXPERIMENTAL PNEUMOCOCCAL HYPOPYON ULCERS IN THE RABBIT

by J. M. Robson & G. I. Scott, *British Journal of Experimental Pathology*, **24**, 50–56, April 1943

Continuing their investigation on the treatment of experimental infections of the eye, the authors produced pneumococcal ulcers in rabbits by injecting a 6 to 15-hour culture of a type 19 pneumococcus into the cornea, so as to raise a small blister under the corneal epithelium. There was some variation in the severity of the resulting lesion, but usually there

was severe ulceration, with iritis and hypopyon. One eye in each rabbit was used as a control. Penicillin was applied to the eye hourly for 36 hours and then hourly by day for about 3 days more.

*Result.* In 28 rabbits in which treatment was started 1, 6 or 12 hours after infection, all the untreated control lesions were classed as severe, whereas 22 of the treated lesions were classed as slight or very slight. Even when treatment was started at 24 hours there was some effect—8 out of 14 treated lesions were slight, while all the control lesions were severe.

[*Reference is made above only to those parts of the original paper in which the use of penicillin is described. A short and more general abstract of the same paper has previously been published* (*BMB* **164**).]

**220**

## CHEMOTHERAPEUTIC EXPERIMENTS WITH THE VIRUSES OF INFLUENZA A, LYMPHOGRANULOMA VENEREUM AND VACCINIA

by C. H. Andrewes, H. King, & M. van den Ende, *Journal of Pathology and Bacteriology*, **55**, 173–181, April 1943

The authors, who are on the staff of the *National Institute for Medical Research*, London, tested the action of penicillin on mice infected with influenza, vaccinia and lymphogranuloma venereum viruses. The penicillin was injected 3-hourly for 72 hours. It had no therapeutic effect in mice with any of these diseases, in spite of the fact that it suppressed the growth of lymphogranuloma venereum virus in tissue culture.

[*This brief abstract refers only to results with penicillin. A fuller summary will appear in a future issue of BMB.*]

**221**

## SYNERGISTIC EFFECT OF PARA-AMINOBENZOIC ACID AND SULPHAPYRIDINE ON PENICILLIN

by J. Ungar, *Nature*, **152**, 245–246, 28/8/43

The author is a member of the research staff of the *Glaxo Laboratories, Ltd.*, Greenford, Middlesex. He found that in a medium containing 0·1 % hydrolyzed casein the addition of *p*-aminobenzoic acid (1 : 1,000–1 : 10,000) raised the figure at which a penicillin solution inhibited *B. subtilis* from 1 : 100 to 1 : 6,000 and *Staph. aureus* from 1 : 40,000 to 1 : 75,000–1 : 100,000. A similar effect was seen in glucose broth with *B. subtilis*. There was no effect with *Strept. hæmolyticus*.

Sulphapyridine in concentrations too low to inhibit growth (1 : 2,000–1 : 50,000) had a similar effect, raising the titre for *Staph. aureus* in digest broth from 1 : 30,000 to 1 : 70,000. A similar effect was shown in glucose broth against the staphylococcus and streptococcus. No further rise in titre occurred when *p*-aminobenzoic acid was added as well as sulphapyridine.

Groups of six mice were injected with staphylococci and streptococci and treated for 4 days with small doses of (i) sulphapyridine, (ii) penicillin, (iii) sulphapyridine + penicillin. With the streptococcus 1 mouse in each of groups (i) and (ii) was alive at 7 days, and 4 in group (iii). With the staphylococcus a similar prolongation of life was shown, though the sulphapyridine alone was entirely without effect. Control mice were all dead on the first day. [The groups of mice in this therapeutic trial are too small for the experiment to be considered entirely conclusive.]

**222**

## INHIBITION OF UREASE BY PENICILLIN

by J. C. Turner, F. K. Heath & B. Magasanik, *Nature*, **152**, 326, 18/9/43

The authors, who are members of the U.S. Army in Great Britain, reported that highly purified penicillin preparations, with an activity of up to 850 Oxford units per mg., inhibited the action of urease. From results with preparations of different activities it was thought probable that the inhibition was a function of penicillin itself and largely independent of the impurities present.

It was suggested that the elevation of blood urea, found by Florey & Florey (1943) to have occurred in some patients treated with penicillin, might be attributable in part to *in vivo* inhibition of urease.

[This suggestion should be accepted with reserve, as it is unlikely that concentrations in the body ever approach those used in these *in vitro* tests.]

REFERENCE

[1] Florey, M. E., & Florey, H. W. (1943) *Lancet*, **1**, 387

[1] [*see BMB* **233**]

**223**

## THE PRODUCTION OF PENICILLIN

by S. W. Challinor & J. MacNaughtan, *Journal of Pathology and Bacteriology*, **55**, 441–446, October 1943

The authors, who are working in the Bacteriology Department and the *Wilkie* Surgical Research Laboratory in the University of Edinburgh, emphasize the difficulty of producing large amounts even of crude penicillin. The mould takes several days to reach maximum production and the yield of penicillin is small. Further, since a shallow layer of culture medium is necessary, large amounts of space are needed for incubation. For these reasons they turned their attention to finding substances which, added to the basic medium, would increase the yield.

The basic medium was the modified Czapek-Dox used by Abraham *et al.* (1941), with 10 % yeast extract. On the assumption that the mould at the height of its activity might produce growth-promoting substances, matured medium (*e.g.* on the 11th day) was added to fresh medium. This accelerated the production of penicillin but reduced its stability, probably because of the acids formed. A better result was obtained by adding buffers or $CaCO_3$ to the medium to maintain the *p*H at about 7. Using a medium of the following composition :

| | | | |
|---|---|---|---|
| $NaNO_3$ | 3·0 g. | $Na_2HPO_4.12H_2O$ | 33·5 g. |
| KCl | 0·5 g. | Glucose (A.R.) | 40·0 g. |
| $MgSO_4.7H_2O$ | 0·5 g. | Yeast extract | 100 ml. |
| $FeSO_4.7H_2O$ | 0·01 g. | Distilled water | to 1000 ml. |
| $KH_2PO_4$ | 6·5 g. | | |

yields in individual cultures were increased 6–8 times, *e.g.* from 1 to 6 Oxford units per cm.³ By adding 5 cm.³ of a 10 % aqueous suspension of $CaCO_3$ to 200 cm.³ of basic medium, an increase was also obtained.

The authors observed that the yield varied according to the strain of *Penicillium notatum* employed. Higher yields were reported by workers using other strains. [And are also obtainable on other media, whose formulæ are as yet unpublished.]

REFERENCE

[1] Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., & Florey, H. W. (1941) *Lancet*, **2**, 177

[1] [*see BMB* **212**]

**224**

## A NEW RAPID METHOD FOR PENICILLIN ASSAY

by U. Wilson, *Nature*, **152**, 475–476, 23/10/43

The present assay methods for penicillin, all of which depend on bacterial inhibition, usually entail overnight incubation. In this communication the author, who is on the staff of the *Wellcome* Physiological Research Laboratories, describes a test which can be read after 3–3½ hours. Aseptic technique is not necessary.

The penicillin solution to be tested was initially diluted so as to contain about 1 unit per cm.³ and then serially diluted with nutrient broth. To tubes containing 1 cm.³ of these broth dilutions were added 0·2 cm.³ of a suspension of 500–700 × $10^6$ organisms of a group A β-hæmolytic streptococcus and 0·8 cm.³ of a 5 % suspension of sheep erythrocytes. After mixing, the tubes were incubated in a waterbath at 37° C. for 3–3½ hours. They were then centrifuged and examined for hæmolysis, absence of hæmolysis indicating a bacteriostatic concentration of penicillin. The streptococcus was grown on plain agar and washed off with broth before use, to avoid the inclusion of preformed hæmolysin.

225

## INHIBITION OF PENICILLIN IN ROUTINE CULTURE MEDIA

by G. J. Harper, *Lancet*, **2**, 569–571, 6/11/43

In making bacteriological investigations into the results of treatment with antiseptics, it is a serious difficulty that traces of antiseptic may be carried over into the cultures and prevent the growth of bacteria which are present. The author of this paper, who is senior technical assistant in the *Medical Research Council* Unit at the Birmingham Accident Hospital, describes a method for removing penicillin when making such cultures. For this purpose he utilised the discovery of Abraham & Chain (1940) that certain bacteria contain an enzyme, penicillinase, which destroys penicillin.

Strains of *Bact. coli*, *Bact. aerogenes*, *Bact. typhosum*, *Bact. typhi-murium*, *Bact. flexneri*, *Ps. pyocyanea*, *Proteus vulgaris*, and an air micrococcus, all inactivated penicillin to a greater or less degree, but the most powerful inactivator was the paracolon bacillus, of which 7 strains isolated from penicillin-treated wounds were all found to destroy penicillin. Living cultures, culture filtrates and dried preparations of this bacillus all appeared to be active. For bacteriological use, the 24-hour growth on a solid medium was suspended in the minimum amount of distilled water and precipitated with acetone. After standing an hour the precipitate was treated for 1–2 hours with fresh acetone and then with two changes of ether. After centrifuging, the precipitate was dried quickly *in vacuo* and stored in sterile tubes. The preparation, dry or suspended in water, remained active for at least several weeks.

One mg. of the dry preparation incorporated in a culture tube or plate was shown to inactivate 200 Oxford units of penicillin, an amount greater than that likely to be accidentally encountered in routine work. The paper contains tables showing the success of this method in obtaining a growth of penicillin-sensitive organisms from swabs of penicillin-treated wounds, which on ordinary media appeared to be sterile.

REFERENCE

[1] Abraham, E. P., & Chain, E. (1940) *Nature*, **146**, 837

[1] [*see BMB* **210**]

226

## THE ACTION OF CHEMOTHERAPEUTIC DRUGS (INCLUDING PROFLAVINE) AND EXCIPIENTS ON HEALTHY TISSUE

by F. R. Selbie & J. McIntosh, *Journal of Pathology and Bacteriology*, **55**, 477–481, October 1943

In the course of testing the effect of antiseptics administered locally the authors injected intramuscularly in mice 5 mg. of an impure preparation of the calcium salt of penicillin [concentration of penicillin not stated], dissolved in a small amount of water. The mice were killed at 3 days. No details of the findings are given but it is stated that muscle necrosis was slight—less than that caused by any other antiseptic tested except the sulphonamides, which produced a similar degree of necrosis.

# *Purification and Investigation of Chemical Properties*

*Communications on this subject are few, and although the chemistry of penicillin is receiving urgent attention on both sides of the Atlantic, full results are not yet available for publication.*

227

## PURIFICATION AND SOME PHYSICAL AND CHEMICAL PROPERTIES OF PENICILLIN

by E. P. Abraham & E. Chain, *British Journal of Experimental Pathology*, **23**, 103–115, June 1942

In this paper from the *Sir William Dunn* School of Pathology, Oxford, the authors report in full their chemical investigations up to the time of publication. They give a method for obtaining a penicillin preparation with an activity of 500 Oxford units per mg., and describe some of its physical and chemical properties. Growth of the mould and assays of antibacterial activity were carried out as previously described (Abraham *et al.*, 1941).

Owing to the instability of penicillin, methods of purification were restricted to those depending on distribution between solvents, adsorption, and reduction. Most of the operations were carried out at temperatures of less than 7° C.

The acid properties of penicillin made it possible to extract the substance with certain organic solvents at *p*H 2 and to re-extract it into water, as a salt, at *p*H 6. Penicillin was extracted from the culture fluid by an equal volume of amyl acetate and then converted to the barium salt (20 units/mg.) in one-fifth of the volume of water. Inactive pigment was removed from the solution by treatment with 5 % of charcoal. The penicillin was then transferred to ether and the ethereal solution was passed through a column of Brockmann alumina. The chromatogram showed the presence of at least five different pigments, the penicillin being associated with a light yellow layer near the top of the column. The material was eluted from this fraction by phosphate buffer at *p*H 7, extracted by ether and converted to the barium salt (70–100 units per mg.). A second chromatogram gave material of 100–150 units per mg. On reduction of this material with aluminium amalgam, keeping the aqueous solution neutral, most of the remaining pigment was adsorbed by the alumina formed. The supernatant contained 80 % of the original activity and yielded, after extraction with amyl alcohol, a barium salt of about 300 units per mg. After three further chromatographic adsorptions from amyl acetate, the final column appeared almost colourless and homogeneous. From this was obtained a barium salt of 450–500 units per mg. Elementary analysis gave the following figures: C 44·3; H 4·8; N 4·2; Ba 22·0.

The barium salt was most stable in aqueous solution between *p*H 5·5 and 7·5, though under these conditions it was rapidly inactivated by heating to 100° C. It was quickly inactivated by dilute acid and alkali at room temperature, but at 0° C. could be kept for one hour at *p*H 2. The free acid was hygroscopic and lost activity readily, but its solution in ether or amyl acetate was stable for some days.

Penicillin was inactivated by: (i) heavy metal cations, such as copper and mercury, (ii) primary alcohols, (iii) ammonia and amines, (iv) hydrazine, hydroxylamine and bisulphite, (v) oxidising agents such as permanganate or hydrogen peroxide.

Inactivation by acid, alkali and by boiling at any *p*H was shown to be accompanied by definite chemical changes. Active penicillin showed no buffering power between *p*H 6 and *p*H 10, but inactivation with acid resulted in the formation of a new titratable group with a *p*K of approximately 7·6. At the same time a substance precipitated by base precipitants was formed. Dilute alkali produced a group with a *p*K of approximately 5·0. It was not possible to produce both these groups. Treatment with alkali after acid, or *vice versa*, resulted in no further change. This suggested that the same part of the molecule was involved in both acid and alkaline inactivation. Inactivation by boiling at any *p*H was shown to be accompanied by the loss of a molecule of carbon dioxide.

REFERENCE

[1] Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N.G., Jennings, M. A., & Florey, H. W. (1941) *Lancet*, **2**, 177

[1] [*see BMB* **212**]

228

## THE SPECTROGRAPHIC EXAMINATION OF PENICILLIN PREPARATIONS

by E. R. Holiday, *British Journal of Experimental Pathology*, **23**, 115–119, June 1942

The author, who is a member of the Medical Unit of the *London* Hospital, examined the ultra-violet absorption spectra of penicillin preparations at various stages of purification (Abraham & Chain, 1942).

With increase in the purity of the penicillin there was a fall in the intensity of absorption. Before the stage of reduction with aluminium amalgam, preparations showed high specific extinction coefficients with two distinct maxima at 278–283 mμ and 375–388 mμ. After the reduction they showed low extinction coefficients with one maximum at shorter wavelengths (246–254 mμ) and a pronounced long wave inflexion (300 mμ). The values for material with an activity of 480 units per mg. were $E^{247m\mu}_{0\cdot1\,\%} = 8\cdot5$ and $E^{300m\mu}_{0\cdot1\%} = 2\cdot3$.

The absorption curves showed that most of the material in crude penicillin preparations was chemically changed during the reduction, but it was not possible to state whether the portion changed included penicillin itself.

The molecular extinction coefficient of the purest material was thought to be too low to allow the presence of substituted aromatic rings, but a polysubstituted hydroaromatic ring structure was considered possible.


REFERENCE

[1] Abraham, E. P., & Chain, E. (1942) *Brit. J. exp. Path.* **23**, 103

[1] [*see BMB* **227**]


229

## PURIFICATION AND CHEMISTRY OF PENICILLIN

by J. R. Catch, A. H. Cook & I. M. Heilbron, *Nature*, **150**, 633–634, 28/11/42

Professor Heilbron and his co-workers at the *Imperial College of Science*, London, describe the purification of penicillin by a modified form of chromatography. Penicillin dissolved in an organic solvent, such as ether or amyl acetate, was passed through a column consisting of a 2·5 % precipitate of an alkaline earth carbonate on silica-gel. The constituents of the crude penicillin were thought to be separated primarily according to the strengths of the component acids.

By this process the authors obtained a penicillin strontium salt having an activity stated to be about 500 Oxford units per mg. The material gave analytical figures corresponding with the formula $C_{24}H_{34}O_{11}NSr$. [It is clear from other work that this is not the correct formula for penicillin.]

The substance was degraded by treatment with dilute acid, alkali, or moist organic bases such as aniline. The products isolated were: (*a*) a colourless water-soluble acid, (*b*) varying quantities of a yellow almost insoluble pigment, (*c*) acetaldehyde. The water-soluble acid afforded ether-soluble and ether-insoluble acids on further hydrolysis. The latter gave a positive ninhydrin reaction and was considered to be formed by the splitting of a peptide bond.

230

## PENICILLAMINE, A CHARACTERISTIC DEGRADATION PRODUCT OF PENICILLIN

by E. P. Abraham, E. Chain, W. Baker & R. Robinson, *Nature*, **151**, 107, 23/1/43

These chemical workers in the *Sir William Dunn* School of Pathology and the *Dyson Perrins* Laboratory, Oxford, collaborating to elucidate the constitution of penicillin, report that solutions of penicillin hydrolysed by N/10 sulphuric acid at 100° C. give a strong ninhydrin reaction. The colour intensities given by numerous preparations run parallel to their antibacterial activities. Hydrolysates of the purest preparations (650 and 1,000 Oxford units/mg.) contained about 59 % of their total nitrogen in the form of amino nitrogen. The authors believe the substance responsible for these results to be a fundamental part of the penicillin molecule, and have named it penicillamine.

Penicillamine was precipitated from solution by mercuric chloride. After decomposing the precipitate, crystalline penicillamine hydrochloride was obtained in 30 % – 40 % yield.

Penicillamine showed no measurable rotation in aqueous solution. It had three titratable groups with *p*K values of 2·0, 7·9 and 10·5. The nitrogen was present as an amino group. Penicillamine gave a deep blue colouration with ferric chloride. It was oxidised by iodine, the reaction being reversible. On heating with *p*-nitro-phenylhydrazine in N hydrochloric acid, glyoxal *p*-nitrophenylosazone was produced in 20 % yield.

231

## PENILLIC ACID, AN OPTICALLY ACTIVE ACID FROM PENICILLIN

by W. M. Duffin & S. Smith, *Nature*, **151**, 251, 27/2/43

The authors, working at the *Wellcome* Physiological Research Laboratories, found that when highly active penicillin preparations were kept in aqueous solution at *p*H 2 there occurred a rise in rotation. Only part of the material could then be extracted by ether, and there was left in the colourless aqueous phase a substance which they named penillic acid. This could be extracted from the aqueous solution by butyl alcohol, from which it could be separated in crystalline condition. The yield was directly proportional to the biological activity of the penicillin used. With the best material it amounted to 20 % of the barium penicillin.

Penillic acid decomposed at 175° C. with evolution of gas. In aqueous solution it had a rotation of + 600 for the mercury green line. It gave a bluish-purple colour with ninhydrin. It was precipitated by mercuric chloride and phosphotungstic acid, and formed a sparingly soluble silver salt. It did not show the blue colouration with ferric chloride found by Abraham *et al.* (1943) to be given by penicillamine.


REFERENCE

[1] Abraham, E. P., Chain, E., Baker, W. & Robinson, R. (1943) *Nature*, **151**, 107

[1] [*see BMB* **230**]


# *Further Clinical Trials*

***The first clinical trials of penicillin were part of a comprehensive investigation which is reported under another heading. The clinical reports described here, although limited by the relative scarcity of penicillin, provide conclusive confirmation of its therapeutic value. The first of the reports below is not comparable in scope with the others, but it is an interesting example of an early clinical application of penicillin.***

232

## ANTISEPTIC SNUFFS

by M. E. Delafield, E. Straker & W. W. C. Topley, *British Medical Journal*, **1**, 145–150, 1/2/41

This paper, by three members of the *Emergency Public Health Laboratory Service*, described the technique used and the results obtained in some preliminary experiments on the control of the bacterial flora of the nose and nasopharynx by means of antiseptics applied locally in the form of a snuff.

The subjects were examined on 3 successive days each week. Two nasal, one pharyngeal and two tonsillar swabs were taken. Each swab was immediately emulsified in 2 cm.³ of nutrient broth, the swab being rotated 20 times and then pressed against the side of the bottle. After this had been repeated 3 times, 0·6 cm.³ of the broth was pipetted into the middle of a sterile Petri dish, and then absorbed on to a sterile 7-cm. filter paper. The paper was transferred to the surface of a blood-agar plate. After contact had been made the paper was removed and the plate was incubated at 37° C. Examinations were made at 24 and 48 hours. After 48 hours the plates were photographed against a dark background with oblique back-lighting. The number of colonies was counted in the prints. The method gave better quantitative results than other methods tried, but the normal variation was found to be large. Growth was usually heaviest from the tonsils and least from the nasal cavities.

Of the three antiseptics used, sulphathiazole (10 % in lycopodium powder or in magnesium carbonate as a diluent) was given the most extensive trial. Proflavine (5 %) was soon found to be less effective and was also æsthetically objectionable. Penicillin was then so scarce that only 4 cases could be treated with it.

The penicillin snuff contained 1 part by weight of penicillin, 5 parts of menthol and 94 parts of lycopodium. [The penicillin used was a crude preparation of the sodium salt containing about 10 Oxford units per milligram. With six applications a day the daily dose of penicillin was about 25 to 35 units.]

*Results.* The 4 cases treated with penicillin were all relatively heavy nasal carriers of staphylococci. The numbers of staphylococci were greatly decreased during treatment. In

3 cases the period of treatment was short (7–10 days), and staphylococci returned after the penicillin was discontinued. The authors point out that, though traces of antiseptic carried over on the swabs could not always be excluded as a cause of the reduced bacterial count, in one case staphylococci were almost abolished for 8 days after a short course of penicillin.

Nineteen cases were treated with sulphathiazole snuff with promising results, but the number of cases was too small to permit a critical evaluation of the method of treatment. The authors conclude that (i) further work on proflavine is not worth while, (ii) penicillin would probably prove particularly effective, (iii) sulphathiazole might be tried in larger doses with better results, and (iv) a mixture of penicillin and sulphathiazole might prove useful.

Treatment of carriers by this method would probably need to be prolonged.

233

## GENERAL AND LOCAL ADMINISTRATION OF PENICILLIN

by M. E. Florey & H. W. Florey, *Lancet*, **1**, 387–397, 27/3/43

Professor Florey and Dr. M. E. Florey report in this paper the first wide investigation of the possibilities of penicillin therapy. Fifteen patients with serious illness were treated by mouth or by intravenous or intramuscular injection and 172 patients were treated by local application.

The principles of clinical use were based on the knowledge already obtained in the laboratory: (i) Because penicillin had been found to be destroyed by boiling, by acids, alkalis and certain heavy metals, by oxidising agents and by enzymes produced by some common air bacteria, it was clear that solutions for clinical use must be freshly made up and protected particularly from changes of *p*H, from heat and from bacterial contamination. (ii) In concentrations suitable for clinical use, penicillin is bacteriostatic, not bactericidal; a concentration sufficient to suppress bacterial growth must therefore be maintained continuously at the site of infection until the physiological defence mechanisms have effectively acted upon the invading organisms. (iii) It was known that penicillin is excreted rapidly by the kidneys; therefore in systemic administration large and frequent doses were necessary to maintain a bacteriostatic concentration continuously in the body. (iv) No toxic effects had been seen after therapeutic doses in mice or men. Leucocytes survived in high concentrations of penicillin. There was therefore no problem of overdosage. (v) It was known that neither tissue extracts, blood, nor pus interfered with the antibacterial action of penicillin.

### Systemic Administration

*By mouth.* As penicillin would have been destroyed by the acid gastric juice it was necessary to give it in "enteric capsules" (gelatine capsules coated with cellulose acetate phthalate, which is soluble in alkali but not in acid) or by duodenal tube. Preliminary trials on a normal subject showed that the penicillin given by capsule was absorbed, as the blood became bacteriostatic for 2 hours and the urine for 7 hours, but the time at which the capsules dissolved was variable. An attempt was made to treat two patients by capsule and duodenal tube, but there was no marked clinical improvement and it is probable that absorption was irregular and incomplete and the dosage too small.

*Parenteral.* Of the remaining 13 cases treated by general administration, 5 received penicillin intramuscularly alone, 5 both intravenously and intramuscularly, and 3 received local injections at the site of infection in addition to injections by one or both of the other routes. Sometimes a continuous intravenous saline drip was established and the penicillin was given intravenously by this means. The usual dose for an adult was 10,000 Oxford units 2-hourly, or 15 or 20,000 units 3-hourly, but the size of the dose was never arbitrary. By a modification of the slide-cell technique of Colebrook, Storer & Wright (1923) the power of the patient's plasma to prevent the growth of the infecting organism was tested at various intervals between one injection and the next, and the dose was so regulated as to maintain bacteriostasis continuously in the plasma. The largest total dose was 4·7 million units to a patient who was treated continuously for a month. The usual total dose for an adult was about 1·5 million units. As little as 0·1 million units brought about the recovery of an infant of 2 months with multiple osteomyelitis.

Of this group of cases 8 had a severe infection with *Staph. aureus*—acute or sub-acute osteomyelitis, pyæmia or septicæmia with, in one case, fulminating cavernous sinus thrombosis—and all were cured, as was also a patient with chronic osteomyelitis in which *Staph. aureus* and *Strept. pyogenes* were combined. A case of meningitis due to a sulphonamide-resistant streptococcus [reported fully by Fleming (1943)] was cured. In a patient infected with a streptothrix and a streptococcus the streptothrix was eliminated. In another case with a similar mixed infection the organisms were not eliminated, but here the dose of penicillin was clearly too small. A case of sub-acute bacterial endocarditis due to *Strept. viridans* improved during treatment but relapsed as soon as treatment was stopped, at the end of a month.

Certain important facts emerged from the study of these cases. Penicillin prevented bacteria multiplying but had no other effect, and the body defences were therefore called on to overcome the residual infection in the usual way; for this reason the temperature fell slowly over 1 to 2 weeks and was not a guide to the success of treatment in the first few days. By contrast, diminution of pain and improvement in spirits and appetite began early and were usually remarkable. Elimination of bacteria ran parallel with recovery. Where bones were involved, radiograms taken during the period of recovery showed a progressive rarefaction which was presumed to be due to the rapid removal of inflammatory products; recalcification took place later without further treatment. In some cases the blood urea rose, but it fell again during or after treatment; the urine did not contain albumen and there was no sign that the rise was due to renal damage. Anæmia improved during treatment without blood transfusion, and there was no evidence of toxic leucopenia. In 5 cases the infecting organism was isolated before and after treatment and tested against penicillin *in vitro*. In 3 cases there was no change in sensitivity; in 2 cases slight resistance had developed to the extent that four times the previous concentration of penicillin was needed to inhibit growth.

### Local Administration

The same principles as for general treatment were applied. Penicillin was known to act in the presence of pus, but being very soluble had to be renewed frequently. A good effect could be expected only if the whole infected area could be reached, but free drainage, which would have removed the penicillin, was not desirable. (The calcium salt of penicillin was used in most cases as it was easier to handle than the sodium salt, which was hygroscopic. The calcium salt was not, however, suitable for intravenous or intramuscular injection in strong solution.)

The cases treated fell into 4 groups:

i. *Mastoid infections.* After a Schwartze mastoidectomy the wound was sewn up completely and a fine rubber tube without lateral holes was inserted through the upper end to the base of the cavity. Penicillin solution, 250 to 500 units per cm.³ was injected 6-hourly, exudate being aspirated through the tube before each injection. Penicillin ointment was smeared on the suture line. Treatment was continued for 7 days. Fourteen out of 16 acute or sub-acute and 5 out of 6 chronic cases healed by primary union. Two of the failures came early in the series and could be attributed to technical defects.

ii. *Eye infections.* Eighty-nine eye infections of a wide variety of types were treated by aqueous drops or vaseline ointment containing 600 to 800 units of penicillin per cm.³ or gram. Among the infecting organisms were *Staph. aureus*, *Staph. albus*, pneumococcus and gonococcus. A large proportion of the cases was cured. Relapse or failure could always be explained by persistence of an insensitive organism, an associated irritative condition, or failure to persevere with treatment.

iii. *Chronic wound sinuses.* Dry penicillin powder was applied to short sinuses. Long sinuses were treated by inserting a catheter as far as possible, injecting penicillin solution (200–500 units/cm.³) and immediately closing the sinus with a rubber bung. The injections were given twice daily for from 10 to 21 days. Sinuses associated with empyema were treated by injecting the solution twice daily and closing the aperture except for an hour before each injection. Nine out of eleven sinuses healed within a month, the remaining

two requiring surgical treatment for a sequestrum and a bronchopleural fistula respectively.

iv. *Various septic conditions.* Among 50 patients with a variety of septic lesions, healing was obtained by similar methods in all but a few, for whom surgical interference was needed to obtain better access or to drain already sterilised abscesses.


REFERENCES

Colebrook, L., Storer, E. J., & Wright, A. E. (1923) *Lancet*, **1**, 365

[1] Fleming, A. (1943) *Lancet*, **2**, 434

[1] [*see BMB* **236**]


234

**PENICILLIN AND PROPAMIDINE IN BURNS : Elimination of Hæmolytic Streptococci and Staphylococci**

by A. M. Clark, L. Colebrook, T. Gibson, M. L. Thomson & A. Foster, *Lancet*, **1**, 605–609, 15/5/43

This paper is by members of the *Medical Research Council* Burns Unit at the *Royal Infirmary*, Glasgow, who report the results of local penicillin therapy in infected burns. The control of sepsis due to hæmolytic streptococci and staphylococci was desirable not only for treatment but also to eliminate the dangers of cross-infection.

*Method.* Penicillin was incorporated in a cream consisting of lanette wax SX 50 g., castor oil 120 cm.$^3$, water 275 cm.$^3$ Sufficient of the calcium salt of penicillin, dissolved in a small amount of water, was added to the cream to give a final concentration of penicillin of 120 units/gram. It was found that the bacteriostatic activity of the cream was retained at room temperature for about 2 weeks. The cream was thickly spread over the burns and renewed 4 times at 48-hour intervals.

*Results.* No toxic effects were observed and the dressings were painless. Healing was rapid except when delayed by large sloughs or gross skin loss. Forty-one out of 54 wounds were freed from hæmolytic streptococci within 5 days. In the remaining cases healing occurred less rapidly on account of adverse circumstances (*e.g.* interference with the dressings). Staphylococci also disappeared, though more slowly. The authors suggest that a greater concentration of penicillin would have been an advantage.

*Propamidine* [1] (an 0·1 % cream) was used in the same way, but it was less effective and there was, further, a danger of toxic absorption. Neither material had any effect on coliform bacilli, *B. proteus*, or *Ps. pyocyanea*, in cases in which these organisms were present.

[1] [*see BMB* **58** *for an account of this new drug.*]

235

**LOCAL THERAPY OF WAR WOUNDS : 1. With Penicillin**

by R. J. V. Pulvertaft, *Lancet*, **2**, 341–346, 18/9/43

The author, who is the Officer Commanding the Central Pathological Laboratory of the Middle East Forces, opens this paper by describing a simple suction apparatus which he considers almost essential for removing pus from septic wounds before applying antiseptic dressings. A glass nozzle connected with a suction bottle emptied by gravity was applied to the wound, while simultaneously saline solution (the author suggests that 0·1 % electrolytic hypochlorite solution might have been better) was run over the wound by gravity through another nozzle connected to a glass reservoir. By this apparatus pockets could be thoroughly cleaned, and smell and soiled dressings could be greatly reduced.

Penicillin, either the calcium or sodium salt, was applied as a dry powder, a spray, or a wet dressing under paraffin gauze. Occasionally the powder caused transient pain. Detailed reports are given of 15 treated cases, all of which had war wounds with severe sepsis. The results were uniformly good. Within one or two days of beginning penicillin applications, the appearance of the wounds improved and discharge stopped. Gram-positive bacteria largely disappeared, any that remained becoming intracellular. In a few days healthy granulations and epithelial growth were seen, at which stage cultures were often sterile. Sometimes gram-negative bacilli increased in numbers, but they did not produce signs of inflammation. One patient with gas gangrene, who was delirious from toxæmia, recovered without any other treatment. In another case a brain abscess was successfully treated by penicillin powder (calcium salt) applied at operation and penicillin solution injected through a catheter for the next 3 days.

In some *in vitro* experiments the author showed that the activity of penicillin was not reduced by *p*-aminobenzoic acid, by filtrates of *Staph. aureus* cultures, or by the filtrates of a culture in which *Ps. pyocyanea* had grown in the presence of penicillin. *S. typhi*, grown in the presence of penicillin, produced filamentous forms, showing that division was arrested ; such forms could not be subcultured and would presumably be readily ingested by phagocytes.

236

**STREPTOCOCCAL MENINGITIS TREATED WITH PENICILLIN : Measurement of Bacteriostatic Power of Blood and Cerebrospinal Fluid**

by A. Fleming, *Lancet*, **2**, 434–438, 9/10/43

This paper reports fully a case of which some notes were given by Florey & Florey (1943). Penicillin was administered intrathecally for the first time in man, in conjunction with intramuscular injections. The detailed observations made by the author established the value of intrathecal injection and led to the treatment of subsequent cases of meningitis by intrathecal injection alone.

The patient was a man of 52 who had been febrile for 7 weeks and had had signs of meningitis for 3 weeks. There had been an early response to sulphapyridine, but afterwards sulphathiazole was without effect. About 6 colonies per cm.$^3$ of a non-hæmolytic streptococcus were isolated from the cerebrospinal fluid 6 and 2 days before penicillin treatment was begun, by the use of " sloppy " glucose agar cultures (0·2 % agar), after ordinary culture methods had failed.

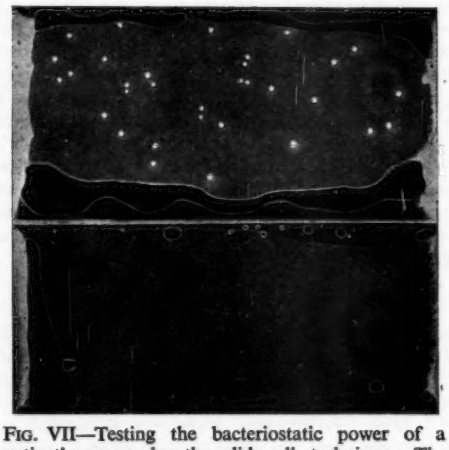


FIG. VII—Testing the bacteriostatic power of a patient's serum by the slide-cell technique. The upper picture shows growth of staphyloccci from an inoculum of a 24-hour broth culture (2·5 mm.$^3$ of a 1:80,000 dilution) in a slide-cell, using the patient's serum *before treatment* as the culture fluid. In the lower picture, the patient's serum *after penicillin treatment* has been used, and the absence of staphylococcal colonies indicates that it has acquired strongly bacteriostatic properties. Photograph reproduced from the *Lancet*.

The patient's serum specifically agglutinated this organism, which was shown *in vitro* to be insensitive to sulphathiazole and sensitive to penicillin, though less so than a sensitive strain of *Staph. aureus*.

When penicillin treatment was started the patient appeared to be moribund. Some improvement occurred on 2-hourly intramuscular injections (usually 10,000 Oxford units), and further improvement when intrathecal injections (usually 5,000 units) were given once daily in addition. On one occasion the penicillin was dissolved in the patient's serum for injection, with the object of providing opsonins. Treatment was continued for 14 days, after which the patient's condition was excellent. Recovery was complete.

By *in vitro* tests the author was able to show that during intramuscular therapy the cerebrospinal fluid had only half

the bacteriostatic power of the blood serum, but that intrathecal injections greatly raised its titre. At the best, 24 hours after an intrathecal injection, the cerebrospinal fluid inhibited *Staph. aureus* at 1 : 160 and the patient's streptococcus at 1 : 40, compared with 1 : 2 and 1 : 1 for the serum 2 hours after an intramuscular injection. The cerebrospinal fluid was coloured yellow by the penicillin.

The bacteriostatic power of the serum was tested by micromethods based on the fact that staphylococci and streptococci will grow as well-defined colonies in human serum, either whole or diluted with saline up to 1 : 100 or more.

i. *Slide-cell cultures.* On a waxed slide were placed 50 mm.³ volumes of the diluting fluid (normal serum diluted, *e.g.* 1 : 10 with saline); 2 : 3 or 1 : 2 serial dilutions of the serum to be tested were made on the slide, 2·5 mm.³ of a diluted culture of staphylococcus or streptococcus was added, and the resulting fluids were run into slide-cells and sealed and incubated.

The bacterial cultures used in this test were so diluted as to produce between 15 and 50 colonies from the 2·5 mm.³ used.

ii. *Capillary tube cultures.* Twenty-five mm.³ of the patient's serum (whole or diluted) mixed with 2·5 mm.³ of culture diluted as above, was run into a capillary tube, which was then sealed and incubated.

iii. *Use of hæmolysis as indicator.* Defibrinated human blood, freed of leucocytes, was added in equal volume to serial dilutions of the patient's serum in saline (25 mm.³ volumes). A loopful of culture of a hæmolytic streptococcus was added to each preparation and the fluids were run into slide-cells (when testing bacteriostasis due to sulphonamides the culture had to be considerably diluted, but this was not necessary with penicillin). After incubation hæmolysis was easily seen wherever the streptococci grew.


REFERENCE

[1] Florey, M. E., & Florey, H. W. (1943) *Lancet*, **1**, 387

[1] [*see BMB* 233]


237

## PENICILLIN IN WAR WOUNDS : A Report from the Mediterranean

[unsigned] *Lancet*, **2**, 742

## THE TREATMENT OF WAR WOUNDS WITH PENICILLIN

by L. P. Garrod, *British Medical Journal*, **2**, 755–756, 11/12/43

These are reviews of "A preliminary report to the War Office and the Medical Research Council on investigations concerning the use of penicillin in war wounds. Carried out under the direction of Professor H. W. Florey and Brigadier Hugh Cairns." This memorandum, published by the War Office in London, has a limited circulation and is not generally available.

Work in England and America had established that among the organisms most sensitive to penicillin were three of those most damaging in war wounds—*Streptococcus pyogenes*, staphylococci and the clostridia. It had further amply established that infections by the pyogenic cocci, however severe, could usually be controlled by penicillin. When penicillin was taken to the Mediterranean theatre of war, therefore, the object was not to establish its effectiveness, but to ascertain the best and most economical methods of use to prevent sepsis in battle casualties. The principles of use were those already established in the laboratory and clinic. As supplies of the drug were extremely limited, attention was always directed to using the smallest amount. In this series, therefore, parenteral injection was reserved for those in whom local application could not be expected to reach all infected areas, although with plentiful supplies many more patients would probably be treated parenterally.

The technique of local application was studied fully. It was not found possible to compare the results with those obtained by current methods of treatment, but it appeared that in the ordinary way large numbers of the more serious war wounds became infected. The effectiveness of the sulphonamides, which were widely used for battle casualties, was still neither established nor disproved.

*Chronic sepsis.* The first patients treated had septic wounds from 3 weeks to 4 months old, none of which had responded to sulphonamides. Considerable success was obtained in soft tissue wounds by local application of penicillin; bacteria disappeared and the wounds became clean. Compound fractures were beyond the effective reach of local applications, but some recovered on parenteral administration. In others, the extensive suppuration was maintained by insensitive organisms after those sensitive to penicillin had been eliminated. It was felt that to treat sepsis at this stage was wasteful of penicillin and of man-power, both military and medical, and the next series was treated at the Forward Base Hospital.

*Recent soft tissue wounds.* Current army practice was to excise the wound at the Casualty Clearing Station and leave it open. At the Forward Base Hospital some of the cleanest wounds were then sutured, but many were allowed to heal by granulation, followed later in some cases by delayed suture or grafting. Nearly all such wounds contained pathogenic organisms, and many became septic. The technique for using penicillin in soft tissue wounds at the Forward Base Hospital was as follows. After conservative excision, the skin in particular being as far as possible preserved, the skin was undercut to mobilise the edges. The wound was closed by deep skin sutures, occasionally with muscle sutures in addition. From 1 to 5 fine rubber tubes were inserted through stab holes or through the incision, reaching to the base of the wound and protruding through the dressings at the free end. Three cm.³ of a 250 unit per cm.³ penicillin solution were injected immediately through a syringe attached to each tube, and thereafter 12-hourly for 4 or 5 days. Many wounds were healed in 10 to 12 days, and by 3 weeks complete healing had taken place in 104 out of 171 cases and incomplete healing (*i.e.* with a small area of granulation in some part of the wound) in 60 more. Only 7 were classified as failures. Pus due to *Ps. pyocyanea*, *B. proteus* or coliform organisms was often formed, but it caused no inflammatory reaction and did not delay healing unless there was a dead space in which it could accumulate.

Even the largest wounds healed completely when treated in this way. There was general agreement that healing was complete in about half the customary time, and that scar formation and permanent disability were very greatly reduced. It is emphasised that under no circumstances should a wound be sewn up in this way in the forward areas, but only in a hospital where the patient can remain.

In another series of patients penicillin-sulphonamide powder, 5,000 units per gram, was insufflated at the Casualty Clearing Station, and suture with tubes, or powder insufflation, was carried out at the Forward Base Hospital. About half the wounds were sterile when received at the Forward Base Hospital; the final result as regards healing was similar to that of the first group.

*Compound fractures.* These were too extensive for local treatment, and were treated by parenteral administration after suture of the wounds. About 100,000 units of penicillin were given daily for the first 3 days and 50,000 for 2 days more. On this dosage the less serious fractures did well, but some failures were recorded, particularly in fractured femur. Out of 31 patients, complete skin union occurred in 16, partial union in 10, and failure in 5. It seemed clear that with a larger dose better results might be achieved, and a total of 700,000–1,000,000 units in 5–10 days for femur and tibia, and of 500,000 units for other fractures, was recommended for future use. Preliminary treatment with penicillin powder at the Casualty Clearing Station was an asset in achieving a good result, and it was recommended that penicillin in the forward areas should be reserved for compound fractures.

*Gas gangrene.* Seven patients were treated parenterally and 4 recovered. In 2 who died the infection had been arrested and death was caused by toxæmia. It was concluded that penicillin should not obscure the necessity for excision of all dead muscle and for giving massive doses of anti-gas-gangrene serum. Its greatest use for gas gangrene would probably be as a prophylactic.

*Head wounds.* As the results of current methods of treating fresh head wounds were very good, penicillin was reserved for penetrating wounds more than 3 days old. Of 23 wounds from 3 to 12 days old, almost all were infected with gram-positive pyogenic organisms and about half were suppurating. The principle of closure with tubes was employed. Twenty cases healed satisfactorily and three died. In one of these

the infection had been controlled, in another only coliform organisms were present at autopsy, and the third, with an 8-day old brain wound containing pneumococcal abscesses, received too little penicillin.

*Other groups of cases.* A few cases of spinal cord injury and of burns infected by sulphonamide-resistant streptococci were treated with good results.

Ten cases of gonorrhœa, 9 of them sulphonamide-resistant, were treated with an arbitrary dose of 12 injections in 48 hours, totalling 180,000 units. Immediate cessation of discharge, " like turning off a tap," was invariable. There was no relapse during the time of observation (2 to 4 weeks).

238

**INFECTED BURNS AND SURFACE WOUNDS : The Value of Penicillin**

by D. C. Bodenham, *Lancet*, **2**, 725–728, 11/12/43

The author, who is officer in charge of a *Royal Air Force* Burns Unit, briefly discusses the infective processes liable to complicate burns and the principles of first aid and hospital treatment. Of 150 major burns a large number were infected with streptococci and nearly all with staphylococci. Streptococci on the surface of a burn, unless sulphonamide-resistant, could be reduced in numbers, though rarely abolished, by sulphonamides. Staphylococci were hardly affected by any sulphonamide except sulphathiazole, and in comparison with penicillin the action of this drug was slight. *Ps. pyocyanea* and *Bacillus proteus*, which are resistant to the sulphonamides and penicillin, were found as secondary invaders of many infected burns. The pus they produced sometimes interfered mechanically with skin grafts, but they did not delay epithelialisation.

Seventy-five burns of a duration of 7 to 180 days were treated with penicillin. All but 6 had previously received sulphonamide treatment. Penicillin eliminated staphylococci and streptococci in all but 3 patients, one of whom was later found to have a sequestrum. The failures were all in the group which was treated 48-hourly. In the larger group treated every 24 hours there were no failures. The swab was negative on the second day in about one-quarter of the infections and within a week in five-sixths.

The author recommends two preparations of penicillin for general use :

i. *Powder.* Sulphanilamide was used as a vehicle. The sulphanilamide, mixed with 5 % by weight of light magnesium oxide to prevent caking, was autoclaved at 1½ atmospheres for 20 minutes, and when cold was shaken with penicillin powder to a final strength of 1,000 units of penicillin per gram. This was applied as a " frosting " to give a concentration of 4 units per cm.² of burn surface.

ii. *Cream.* Equal parts of soft paraffin, lanette wax SX and water were mixed at 60° C. and autoclaved in half-filled jars with the cap on at 1½ atmospheres for 30 minutes. During cooling the cream was shaken several times. When cool, an aqueous solution of penicillin containing 500 units per cm.³ was stirred in, to make a final strength of 100 units per gram. This also was applied to the burn so as to give a concentration of 4 units per cm.² [A similar cream consisting of 30 % lanette wax in water has been found satisfactory in a warm climate.]

The cream was applied every 24 hours, or every 48 hours if the surface was relatively dry. The powder was used every 24 hours if a dry application was especially desirable, but it disappeared from the burn surface more quickly than the cream and appeared therefore to be somewhat less effective.

# APPENDIX

239

**THE OXFORD UNIT**

The potency of penicillin preparations can at present be measured only by biological assay. Assay is carried out *in vitro* against a culture of a sensitive organism. As biological methods are subject to many variables it is necessary that each assay should be a comparative measurement against a standard of known potency. Early in the work at Oxford, for the convenience of workers inside the laboratory, a purely arbitrary penicillin standard was adopted by which the unit was defined as that amount of penicillin contained in 1 cm.³ of a certain buffer solution.

Dry preparations of partially purified penicillin have since been standardised against the original solution, and the " Oxford unit " (which has also been described as the " Florey unit ") has been adopted as the standard of measurement by most workers in Britain, the U.S.A. and Canada. The potency of the unit is such that 0·01–0·02 of a unit per cm.³ inhibits the growth of 26 sensitive strains of the staphylococcus.

240

**THE SLIDE-CELL TECHNIQUE**

This technique was described by Sir Almroth Wright for measuring the bacteriostatic power of blood and serum. It depends on the fact that certain bacteria, particularly staphylococci, when planted in blood or serum in suitable numbers, will grow out as discrete colonies which can be counted under the low power of the microscope. In the original test (Colebrook, Storer & Wright, 1923) five narrow strips of paper about 0·1 mm. thick, which have been dipped in hot vaseline, are laid across a sterile microscope slide and another sterile slide is placed on top. This forms four chambers of a depth of about 0·1 mm. and a capacity of about 50 mm.³ The samples of blood or serum, mixed with suitable dilutions of culture, are run into the chambers and the ends are sealed with wax. After incubation the colonies are counted. Fleming has made much use of this technique in testing the effects of antiseptics on bacteria and leucocytes. An example of its use in assessing the bacteriostatic power of the blood of patients undergoing penicillin treatment is given elsewhere.[1] It should be noted that serum, not whole blood, should be used in testing for antibacterial power due to penicillin as the erythrocytes take up less than 10 % of the penicillin in the blood (Rammelkamp & Keefer, 1943b)[2] and leucocytes, if present, would destroy some of the bacteria.

The modified test mentioned by Florey & Florey (1943)[3] was designed to detect bacteriostasis in a single drop of the patient's serum. A measured drop (5–10 mm.³) is placed directly on one end of a sterile slide, which already bears a row of drops of the same volume of normal serum. Serial dilutions are made, the last drop of normal serum being left as a control. A small coverslip is put on to each drop and the edge is sealed with wax. After incubation the colonies are counted and the number is compared with that in the control. Where there is a fully bacteriostatic concentration of penicillin, no colonies develop.

[1] [*see BMB* **236**] [2] [*see BMB* **242**] [3] [*see BMB* **233**]

241

**COMMUNICATIONS ON PENICILLIN PUBLISHED IN BRITAIN BUT NOT REVIEWED IN THIS NUMBER**

[*Editorials and papers on other subjects in which penicillin is briefly mentioned have been omitted.*]

I. *Work more fully reported in Other Papers reviewed Elsewhere in this Number as indicated in Square Brackets*


Abraham, E. P., Baker, W., Chain, E., Florey, H. W., Holiday, E. R. & Robinson, R. (1942) Nitrogenous character of penicillin. *Nature*, **149**, 356 [*see BMB* **230**]

Abraham, E. P. & Chain, E. (1942) Purification of penicillin. *Nature*, **149**, 328 [*see BMB* **227**]

Challinor, S. W. (1942) Production of penicillin. *Nature*, **150**, 688 [*see BMB* **223**]

Fleming, A. (1942) A simple method of using penicillin, tellurite, and gentian violet for differential culture. *Brit. med. J.* **1**, 547 [*see BMB* **200**]

Robson, J. M. & Scott, G. I. (1942) Effect of certain chemotherapeutic agents on experimental eye lesions produced by *Staphylococcus aureus*. *Nature*, 149, 581 [see *BMB* 218, 219]

II. *Correspondence in the Medical Press*

Bacharach, A. L. (1943) Penicillin. *Brit. med. J.* 2, 434 [On chemical research]

Fleming, A. (1941) Penicillin. *Brit. med. J.* 2, 386 [On his discovery]

Maclean, I. H. (1940) Penicillin. *Lancet*, 2, 345 [Isolation of *H. pertussis*]

III. *Mention of Penicillin in Discussions, Lectures and Reviews*

Allcroft, R. (1942) Antibacterial agents derived from micro-organisms with special reference to gramicidin. *Vet. Bull.* 12, R1

Biochemical Society (1942) The mode of action of chemotherapeutic agents. A discussion. [Contributions by Fleming, Chain & Abraham.] *Biochem. J.* 36, 1; *Lancet* (1941) 2, 761

Dale, Sir Henry (1943) A prospect in therapeutics (Frederick Price Lecture to the Royal College of Surgeons, Edinburgh). *Brit. med. J.* 2, 411

Dawson of Penn, Lord (1943) [Speech on medical research, in the House of Lords]. *Brit. med. J.* 2, 186

Fleming, A. (1931) Some problems in the use of antiseptics. *Brit. dental J.* 52 ii, 105

Fleming, A. (1936) Selective bacteriostasis. Opening paper of a discussion on the inhibitory action on the growth of bacteria and fungi of (1) substances of known constitution and (2) products of the growth of micro-organisms. Importance in the preparation of selective culture media. *Proc. Second Int. Congress of Microbiology*, London, 1937, page 33

Fleming, A. (1940) Antiseptics in wartime surgery. *Brit. med. J.* 2, 715

Fleming, A. (1941) Chemotherapy and wound infection. *Proc. roy. Soc. med.* 34, 342

Garrod, L. P. (1943) Recent advances in the antiseptic treatment of wounds. *Brit. med. Bull.* 1, 26 [*BMB* 48]

Mellanby, Sir Edward (1943) Medical research in wartime. [Ludwig Mond Lecture, Manchester University.] *Brit. med. J.* 2, 351

Miles, A. A. (1941) Some problems of wound infection. *Lancet*, 2, 507

*Royal Society of Medicine* (1943) Section of experimental medicine and therapeutics. Discussion on penicillin. *Lancet*, 2, 638; *Brit. med. J.* 2, 654 [These are both brief reports of the Discussion. A fuller report will be published in the *Proceedings of the Royal Society of Medicine* early in 1944.]

IV. *Official Announcement*

Medical Research Council (1943) Supplies and distribution of penicillin. *Lancet*, 2, 276; *Brit. med. J.* 2, 274

242

## COMMUNICATIONS ON PENICILLIN PUBLISHED IN OTHER COUNTRIES

*Nearly all these references are to papers published in the U.S.A. As a result of the kind co-operation of Dr. Joseph W. Ferrebee and Dr. Hamilton Southworth, representatives in London of the U.S. Committee of Medical Research, a list of U.S. papers on penicillin, provided by the American Medical Association, was received shortly before this number went to press. This included some titles which could not otherwise have been incorporated. Review articles have not been listed, except when in a language other than English. The present disturbance of communications has made it impossible to ensure that this list is complete in respect of all countries; it is possible that some papers published elsewhere than in the U.S.A. may have been omitted. The Editor would be grateful for information on any significant omissions.*

I. *Bacteriological Applications*

Bornstein, S. (1940) Action of penicillin on enterococci and other streptococci. *J. Bact.* 39, 383

II. *Laboratory Studies on Penicillin and its Biological Properties*

Atkinson, N. (1943a) Antibacterial substances produced by microorganisms, with special reference to those produced by moulds. *Med. J. Austral.* 1, 359

Atkinson, N. (1943b) Antibacterial substances produced by moulds; the antibacterial substances produced by some common penicillia. *Austral. J. exp. Biol. med. Sci.* 21, 15

Bergy, G. A. (1943) Penicillin, and other mold derivatives. *West Virginia med. J.* 39, 272

Birkinshaw, J. H. & Raistrick, H. (1943) Notatin; an antibacterial glucose aerodehydrogenase from *Penicillium notatum* Westling. *J. biol. Chem.* 148, 459

Clifton, C. E. (1943) Large-scale production of penicillin. *Science*, 98, 69

Foster, J. W. (1942) Quantitative estimation of penicillin. *J. biol. Chem.* 144, 285

Foster, J. W. & Woodruff, H. B. (1943a) Improvement in the cup assay for penicillin. *J. biol. Chem.* 148, 723

Foster, J. W. & Woodruff, H. B. (1943b) Microbiological aspects of penicillin. I. Methods of assay. *J. Bact.* 46, 187

Foster, J. W. & Wilker, B. L. (1943) Microbiological aspects of penicillin. II. Turbidimetric studies on penicillin inhibition. *J. Bact.* 46, 377

Hac, L. R. & Hubert, A. C. (1943) Penicillin in treatment of experimental *Clostridium welchii* infection. *Proc. Soc. exp. Biol. N.Y.* 53, 61

Hamre, D. M., Rake, G., McKee, C. M. & MacPhillamy, H. B. (1943) The toxicity of penicillin as prepared for clinical use. *Amer. J. med. Sci.* 206, 642

Heilman, D. H. & Herrell, W. E. (1942) Comparative antibacterial activity of penicillin and gramicidin: tissue culture studies. *Proc. Mayo Clin.* 17, 321

Herrell, W. E. & Heilman, D. (1943a) Tissue culture studies on cytotoxicity of bactericidal agents. I. Effects of gramicidin, tyrocidine and penicillin on cultures of mammalian lymph node. *Amer. J. med. Sci.* 205, 157

Herrell, W. E. & Heilman, D. (1943b) Tissue culture studies on cytotoxicity of bactericidal agents. III. Cytotoxic and antibacterial activity of gramicidin and penicillin; comparison with other germicides. *Amer. J. med. Sci.* 206, 221

Hirsch, J. (1943) Penicillium notatum Fleming in bakteriyostatik tesirle beraber glukozu okside eden enzym ifrayi. *Istanbul Seririyati*, 25, 151

Hobby, G. L., Meyer, K. & Chaffee, E. (1942a) Activity of penicillin *in vitro*. *Proc. Soc. exp. Biol. N.Y.* 50, 277

Hobby, G. L., Meyer, K. & Chaffee, E. (1942b) Observations on the mechanism of action of penicillin. *Proc. Soc. exp. Biol. N.Y.* 50, 281

Hobby, G. L., Meyer, K. & Chaffee, E. (1942c) Chemotherapeutic activity of penicillin. *Proc. Soc. exp. Biol. N.Y.* 50, 285

Kocholaty, W. (1942) Cultural characteristics of *Penicillium notatum* in relation to the production of antibacterial substance. Indication of the dual nature of the antibacterial substance. *J. Bact.* 44, 469

Lawrence, C. A. (1943) Sterility test for penicillin. *Science*, 98, 413

Lochhead, A. G. & Timonin, M. (1943) The effect of glucose on penicillin potency tests. *Canad. publ. Hlth. J.* 34, 236

McKee, C. M. & Houck, C. L. (1943) Induced resistance to penicillin of cultures of staphylococci, pneumococci and streptococci. *Proc. Soc. exp. Biol. N.Y.* 53, 33

McKee, C. M. & Rake, G. (1942a) Biological experiments with penicillin. *J. Bact.* 43, 645

McKee, C. M. & Rake, G. (1942b) Activity of penicillin against strains of pneumococci resistant to sulfonamide drugs. *Proc. Soc. exp. Biol. N.Y.* 51, 275

Meyer, K., Chaffee, E., Hobby, G. L., Dawson, M. H., Schwenk, E. & Fleischer, G. (1942) On penicillin. *Science*, 96, 20

Meyer, K., Hobby, G. L. & Chaffee, E. (1943) On esters of penicillin. *Science*, 97, 205

Meyer, K., Hobby, G. L. & Dawson, M. H. (1943) The chemotherapeutic effect of esters of penicillin. *Proc. Soc. exp. Biol. N.Y.* 53, 100

Powell, H. M. & Jamieson, W. A. (1942a) Response of sulfonamide-fast pneumococci to penicillin. *Proc. Soc. exp. Biol. N.Y.* 49, 387

Powell, H. M. & Jamieson, W. A. (1942b) Penicillin chemotherapy of mice infected with *Staphylococcus aureus*. *J. Indiana med. Ass.* 35, 361

Rake, G., McKee, C. M. & Jones, H. (1942) A rapid test for the activity of certain antibiotic substances. *Proc. Soc. exp. Biol. N.Y.* 51, 273

...kamp, C. H. (1942) A method for determining the ...ntration of penicillin in body fluids and exudates. *...oc. Soc. exp. biol. N.Y.* **51**, 95

Rammelkamp, C. H. & Bradley, S. E. (1943) Excretion of penicillin in man. *Proc. Soc. exp. Biol. N.Y.* **53**, 30

Rammelkamp, C. H. & Helm, J. D. (1943) Excretion of penicillin in bile. *Proc. Soc. exp. Biol. N.Y.* **54**, 31

Rammelkamp, C. H. & Keefer, C. S. (1943a) The absorption, excretion and toxicity of penicillin administered by intrathecal injection. *Amer. J. med. Sci.* **205**, 342

Rammelkamp, C. H. & Keefer, C. S. (1943b) The absorption, excretion, and distribution of penicillin. *J. Clin. Invest.* **22**, 425

Rammelkamp, C. H. & Keefer, C. S. (1943c) Penicillin: its antibacterial effect in whole blood and serum for the hemolytic streptococcus and *Staphylococcus aureus*. *J. Clin. Invest.* **22**, 649

Rammelkamp, C. H. & Maxon, T. (1942) Resistance of *Staphylococcus aureus* to the action of penicillin. *Proc. Soc. exp. Biol. N.Y.* **51**, 386

Rao, S. S. & De, S. P. (1943) Penicillin. *Current Science*, **12**, 209

Reid, R. D. (1935) Some properties of a bacterial-inhibitory substance produced by a mold. *J. Bact.* **29**, 215

Roberts, E. C., Cain, C. K., Muir, R. D., Reithel, F. J., Gaby, W. L., Van Bruggen, J. T., Homans, D. M., Katzman, P. A., Jones, L. R. & Doisy, E. A. (1943) Penicillin B, an antibacterial substance from *Penicillium notatum*. *J. biol. Chem.* **147**, 47

Robinson, H. J. (1943) Toxicity and efficacy of penicillin. *J. Pharm. exp. Therap.* **77**, 70

Schmidt, L. H. & Sesler, C. L. (1943) Development of resistance to penicillin by pneumococci. *Proc. Soc. exp. Biol. N.Y.* **52**, 353

Schnitzer, R. J., Camagni, L. J. & Buck, M. (1943) Resistance of small colony variants (G-forms) of a staphylococcus towards the bacteriostatic activity of penicillin. *Proc. Soc. exp. Biol. N.Y.* **53**, 75

Smith, L. D. & Hay, T. (1942) The effect of penicillin on the growth and morphology of *Staphylococcus aureus*. *J. Franklin Inst.* **233**, 598

Smith, L. de Spain (1942) The bacteriostatic agent of *Penicillium chrysogenum*. *J. Franklin Inst.* **234**, 396

Taylor, H. G. (1943) Growth of *Penicillium notatum* on various media and the development of an antibacterial substance. *Proc. Soc. exp. Biol. N.Y.* **52**, 299

Tillett, W. S., Cambier, M. J. & Harris, W. H. (1943) Sulfonamide-fast pneumococci. A clinical report of two cases of pneumonia together with experimental studies on the effectiveness of penicillin and tyrothricin against sulfonamide-resistant strains. *J. clin. Invest.* **22**, 249

T'ung, T. (1943) Concentration and preservation of crude penicillin. *Proc. Soc. exp. Biol. N.Y.* **54**, 103

Van Bruggen, J. T., Reithel, F. J., Cain, C. K., Katzman, P. A. & Doisy, E. A. (1943) Penicillin B; preparation, purification and mode of action. *J. biol. Chem.* **148**, 365

III. *Clinical Reports*

Blake, F. G. & Craige, B. (1943) Penicillin in suppurative disease of the lungs. *Yale J. Biol. & Med.* **15**, 507

Bordley, J. E., Crowe, S. J., Dolowitz, D. A. & Pickrell, K. L. (1942) The local use of sulfonamides, gramicidin (tyrothricin) and penicillin in otolaryngology. *Ann. Otol. Rhinol. & Laryngol.* **51**, 936

Dawson, M. H., Hobby, G. L., Meyer, K. & Chaffee, E. (1943) Penicillin as a chemotherapeutic agent. *Ann. intern. Med.* **19**, 707

Guerra y Perez Carral, F. (1943) La quimioterapia por microorganismos. La penicillina en el tratamiento de las endocarditis. *Arch. Latino-Amer. Cardiol. Hematol.* **13**, 25

Herrell, W. E. (1943) Further observations on the clinical use of penicillin. *Proc. Staff Meet. Mayo Clin.* **18**, 65

Herrell, W. E., Cook, E. N. & Thompson, L. (1943) Use of penicillin in sulfonamide-resistant gonorrheal infections. *J. Amer. med. Ass.* **122**, 289

Herrell, W. E., Heilman, D. H. & Williams, H. L. (1942) The clinical use of penicillin. *Proc. Staff Meet. Mayo Clin.* **17**, 609

Herrell, W. E. & Nichols, D. R. (1943) The calcium salt of penicillin. *Proc. Staff Meet. Mayo Clin.* **18**, 313

Keefer, C. S., Blake, F. G., Marshall, E. K., Lockwood, J. S. & Wood, W. B. (1943) Penicillin in the treatment of infections. A report of 500 cases. Statement by the Committee on Chemotherapeutic and other Agents, Division of Medical Sciences, N.R.C. *J. Amer. med. Ass.* **122**, 1217

Likely, D. S. & Swirsky, M. Y. (1943) *Staphylococcus aureus* septicemia treated with penicillin with report of drug side effects. *J. Amer. med. Ass.* **123**, 956

Lyons, C. (1943) Penicillin therapy of surgical infections in the U.S. Army; a report. *J. Amer. med. Ass.* **123**, 1007

Mahoney, J. F., Ferguson, C., Buchholtz, M. & Van Slyke, C. J. (1943) The use of penicillin sodium in the treatment of sulfonamide-resistant gonorrhea in men; a preliminary report. *Amer. J. Syph.* **27**, 525

Pilcher, C. & Meacham, W. F. (1943) The chemotherapy of intracranial infections; the treatment of experimental staphylococcic meningitis with intrathecal administration of penicillin. *J. Amer. med. Ass.* **123**, 330

Robinson, G. H. & Wallace, J. E. (1943) An inoculated penicillin dressing. *Science*, **98**, 329

Sallmann, L. von (1943) Penicillin and sulfadiazine in treatment of experimental intraocular infection with pneumococcus. *Arch. Ophthal. Chicago*, **30**, 426

IV. *Reviews in Languages other than English*

Bonstein, I. (1943) La pénicilline, un puissant extrait bactériolytique. *Rev. méd. Suisse rom.* **63**, 206

Dufresne, R. (1943) La Pénicilline. *Union méd. Can.* **73**, 1202 [Editorial]

Fleming, A. (1942) Penicillina. *Prensa méd. Argent.* **29**, 1163

Garcia de Jalon, P. (1943) Sustancias antibacterianas naturales; penicillina. *Farmacol. Terapéut.* **4**, 131

Lentino, A. S. (1943) El penicillin, un nuevo agente contra la infección bacteriana. *Bol. Inst. Clin. Quir.* **19**, 490

Tolovi, J. (1943) Penicilina. *Rev. Quím. Farm.* **8**, 5

Uzman, L. (1942–3) Penicillin. *Istanbul Seririyati*, **24**, 68; **25**, 6

V. *Official Announcement*

Richards, A. N. (1943) Penicillin. Statement released by the Committee on Medical Research. *J. Amer. med. Ass.* **122**, 235

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Readers of the *Bulletin* should note that supplies of penicillin are not at present available

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